Abstract Book

5th European Congress of Immunology

Editors:

ECI2018 Congress President
Prof. Dr. Marieke van Ham
Head Dept. of Immunopathology
Sanquin Research and SILS, Faculty of Science, University of Amsterdam
Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands
m.vanham@sanquin.nl

ECI2018 Scientific Program Committee President
Prof Dr. Jaques Neefjes
Leiden University Medical CenterDepartment of Cell & Chemical Biology
P.O. Box 9600 , 2300 RC Leiden, The Netherlands
j.j.c.neefjes@lumc.nl

ECI2018 Local Organizing Committee President
Janneke N. Samsom, PhD
Erasmus University Medical Center
Laboratory of Pediatric Gastroenterology
P.O. Box 2040, 3000 CA Rotterdam, The Netherlands
j.samsom@erasmusmc.nl

ECI2018 and EFIS President's Office
Christina Helbig, PhD
Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands
helbig@efis.org

Graphic Design: Christina Helbig

Coordination:

Wiener Medizinische Akademie GmbH
Alser Strasse 4, 1090 Vienna, Austria
+43 1 405 13 83 30
eci2018@medacad.org
www.eci2018.org

GUIDELINES TO READ THE ABSTRACT BOOK

The scientific program is divided into 5 main topic tracks:

Track A: Immune development and differentiation
Track B: Tumor immunology and therapy
Track C: Autoimmunity, allergy and transplantation
Track D: Infections and microbial immune regulation
Track E: Immunomics – Technical advances and big data

There are 10 types of sessions generated from invited and submitted abstracts:

Keynote Lectures (KL): oral presentations by invited keynote speakers
Symposia (S): oral presentations by invited speakers
Joint Symposium (JS): oral presentations by invited speakers
Educational Sessions (EDU): oral presentations by invited speakers
Men and Women in Immunology (MWI): oral presentations by invited speakers
EFIS President's Symposium (EP): oral presentations by invited speakers
Bright Sparks Workshop (BS): oral presentations from selected abstracts
Workshop (WS): oral presentations from selected abstracts
Late Breaking Hot Topics (HT): oral presentations from selected abstracts
Guided Poster Session (P): poster presentations from selected abstracts

How to read the presentation numbers – for example: WS.B1.06.04

WS.B1.06.04: **WS** stands for one of the following session types

KL:	Keynote Lecture	EP:	EFIS President's Symposium
S:	Symposium	BS:	Bright Spark
JS:	Joint Symposium	WS:	Workshop
EDU:	Educational Session	HT:	Late Breaking Hot Topic
MWI:	Men and Women in Immunology	P:	Poster Session

WS.B1.06.04: **B** stands for one of the following tracks:

A:	Immune development and differentiation
B:	Tumor immunology and therapy
C:	Autoimmunity, Allergy and Transplantation
D:	Infections and microbial immune regulation
E:	Immunomics - Technical advances and big data

WS.B1.06.04: **1** indicates the subtopic within the respective track

WS.B1.06.04: **06** indicates the chronological order of sessions within the respective subtopic

WS.B1.06.04: **04** indicates the chronological order of presentations within the respective session

Thus, WS.B1.04.04 indicates the fourth talk in workshop 04 of subtopic B1!

ANNOTATIONS

In the following we are publishing the abstracts as submitted by the authors.
Missing session numbers represent sessions with no abstracts associated. Missing presentation numbers represent withdrawn or embargoed abstracts which have not been received as per date of publication.

COMMITTEES

STEERING COMMITTEE

Marieke VAN HAM, President
René VAN LIER, EFIS President
Jacques NEEFJES, ECI 2018 SPC President
Janneke N. SAMSOM, ECI 2018 LOC President
Reina MEBIUS, NVVI
Barbara BOHLE, ÖGAI
Hans YSSEL, SFI
Jürgen WIENANDS, DGfI
Angela SANTONI, SIICA
Anna FOGDELL-HAHN, SSI
África GONZÁLEZ-FERNÁNDEZ, SEI
Günnur DENIZ, TSI
Peter OPENSHAW, BSI
Blanka RIHOVA, CIS
Anna ERDEI, MIT
Luljeta AHMETAJ, KAAIA
Antonio LANZAVECCHIA, SSAI
Mira BARDA-SAAD, IIS

LOCAL ORGANIZING COMMITTEE

Janneke N. SAMSOM, LOC President
Jacques NEEFJES , SPC President)
Marieke VAN HAM, Congress President
Gerd BOUMA, VUMC
Jan DAMOISEAUX, UMCM
Esther DE JONG, AMC
Christina HELBIG, SANQUIN
Frits KONING, LUMC
Jon LAMAN, UMCG
Yang LI, UMCG
Reina MEBIUS, VUMC
Mihai NETEA, UMCN
Jaap VAN DISSEL, RIVM
Marjolein VAN EGMOND, VUMC
Cees VAN KOOTEN, LUMC
Hans WESTERHOFF, UVA
Gertjan WOLBINK, READE
Nico WULFFRAAT, UMCU

SCIENTIFIC PROGRAM COMMITTEE

Jacques NEEFJES, SPC President
Janneke N. SAMSOM, LOC President
Marieke VAN HAM, Congress President
Ariel AMIRAM, IIS
Silke APPEL, SSI
Flavia BAZZONI, SIICA
Edit BUZÀS, MIT
Günnur DENIZ, TSI
Gary ENTRICAN, BSI
Niels HELLINGS, BIS
Michael LOHOFF, DGfI
Burkhard LUDEWIG, SSAI
Laura PAJAZITI, KAAIA
Vito PISTOIA, SIICA
Angel PORGADOR, IIS
Ricardo PUJOL-BORRELL, SEI
Abelhadi SAOUDI, SFI
Güher SARUHAN-DIRESKENELI, TSI
Anna SEDIVA, CIS
Rene TOES, NVVI
Rudolf VALENTA, ÖGAI
Véronique WITKO-SARSAT, SFI

FINANCE COMMITTEE

Winfried PICKL (EFIS Treasurer)
Clemens SCHEINECKER, ÖGAI
Paul GUGLIELMI, SFI
Carsten WATZL, DGfI
Francesco ANNUNZIATO, SIICA
Rene TOES, NVVI
Arno HANNINEN, SSI
Manel JUAN, SEI
Arzu ARAL, TSI
Lindsay NICHOLSON, BSI

EFIS BOARD

René A.W. VAN LIER, President
Andreas RADBRUCH, President-elect
Lorenzo MORETTA, Past President
Pablo Engel, Secretary General
Winfried PICKL, Treasurer

ABSTRACT REVIEWERS

The organizers of the 5th European Congress of Immunology would like to extend their special thanks to all abstract reviewers for the time they dedicated to the success of the congress:

Luljeta N. Ahmetaj	Kosovo
Francesco Annunziato	Italy
Silke Appel	Norway
Amiram Ariel	Israel
Mira Barda-Saad	Israel
Robert Benson	United Kingdom
Barbara Bohle	Austria
Gerd Bouma	Netherlands
Panagiota Boura	Greece
Elizabeth Brint	Ireland
Sophie Brouard	France
Milan Buc	Slovakia
Edit Buzas	Hungary
Marco Cassatela	Italy
Gil Castro	Portugal
Nathalie Cools	Belgium
Jan Damoiseaux	Netherlands
Tigran Davtyan	Armenia
Esther de Jong	Netherlands
Raffaele De Palma	Italy
Jocelyne Demengeot	Portugal
Joke den Haan	Netherlands
Deniz Gunnur	Turkey
Diana Dudziak	Germany
Gary Entrican	United Kingdom
Anna Erdei	Hungary
Fogdell-Hahn Anna	Sweden
Luke Foster	United Kingdom
Juan Carcia Vallejio	Netherlands
Valbona Gashi	Kosovo
Africa González-Fernández	Spain
Christina Helbig	Netherlands
Niels Hellings	Belgium
Jochen Hühn	Germany
Hanna Jarva	Finland
Régis Josien	France
Adam Klocperk	Czech Republic
Edward Knol	Netherlands
Frits Koning	Netherlands
Jon Laman	Netherlands
Roland Lang	Germany
Ed Lavelle	Ireland
Claude Leclerc	France
Katarzyna Lisowska	Poland
Michael Lohoff	Germany
Burkhard Ludewig	Switzerland
Miodrag Lukic	Serbia
Andreea Lupu	Romania
Gayane Manukyan	Armenia
Reina Mebius	Netherlands
Attila Mocsai	Hungary
Silvia Monticelli	Switzerland
Jacques Neefjes	Netherlands
Mihai Netea	Netherlands
Peter Openshaw	United Kingdom
Barbaros Oral	Turkey
Trevor Owens	Denmark
Laura Pajaziti	Kosovo
Mariana Pavel Tanasa	Romania
Pärt Peterson	Estonia
Vito Pistoia	Italy
Bojan Polic	Croatia
Angel Porgador	Israel
Wilfried Posch	Austria
Ricardo Pujol-Borrell	Spain
Alexander Rosenkranz	Austria
Duygu Sag	Turkey
Janneke Samsom	Netherlands
David Sancho	Spain
Angela Santoni	Italy
Abdelhadi Saoudi	France
Alexandros Sarantopoulos	Greece
Güher Saruhan-Direskeneli	Austria
Sinisa Savic	United Kingdom
Anna Sediva	Czech Republic
Bruno Silva-Santos	Portugal
Ivana Stojanovic	Serbia
Birgit Strobl	Austria
Zoltan Szekanecz	Hungary
Rene Toes	Netherlands
Raivo Uibo	Estonia
Rudolf Valenta	Austria
Jaap van Dissel	Netherlands
Marjolein van Egmond	Netherlands
Jo van Ginderachter	Belgium
Marieke van Ham	Netherlands
Yvette van Kooyk	Netherlands
René van Lier	Netherlands
Sandra van Vliet	Netherlands
Marc Veldhoen	Portugal
Maria Luisa Villar	Spain
Felix M. Wensveen	Croatia
Jürgen Wienands	Germany
Doris Wilfingseder	Austria
Véronique Witko-Sarsat	France
Jacek M. Witkowski	Poland
Gertjan Wolbink	Netherlands
Nico Wulfraat	Netherlands
Hans Yssel	France

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

KEYNOTE LECTURES

KEYNOTE LECTURES

KL.01 Serendipities of acquired immunity

KL01.1
Serendipities of acquired immunity

***T. Honjo**;*
Kyoto University Institute for Advanced Study, Kyoto, Japan.

In 1992, we started working on PD-1 and found that this acts as a brake in the immune system. Then, in 2002, we discovered that PD-1 inhibition could be effective in treating cancer in animal models. After 22 years of study, this idea has borne fruit in a new, breakthrough immunotherapy that is being hailed as a 'penicillin moment' in cancer treatment. I believe that, just as a number of antibiotics developed in the wake of the discovery of penicillin now protect humans against threats of infectious diseases, this discovery will play a leading role in advancement of cancer immunotherapy so that in the future the fear of dying from cancer will cease to exist. Through evolution, vertebrate animals have developed immunity against infection by microorganisms. In the process, they incidentally acquired a sophisticated system for diversifying genomic information by combining gene fragments. It was doubly fortunate that the success in cancer treatment via PD-1 inhibition brought the realization that immunity, a "weapon" against infectious diseases, could also serve as a "shield" against cancer. It has been said that, whereas humankind's greatest enemies in the 20th century were infectious diseases, cancer is the major foe in the 21st century. It is a pleasant surprise to discover that the acquired immunity system holds the keys to overcoming both of these difficult medical challenges.

KL.02 Trained immunity: reprogramming innate immunity to protect against infections

KL02.1
Trained immunity: reprogramming innate immunity to protect against infections

***M. G. Netea**;*
Radboud University Medical center, Nijmegen, Netherlands.

The inability of innate immunity to build an immunological memory, considered one of the main characteristics differentiating it from adaptive immunity, has been recently challenged by studies in plants, invertebrates, and mammals. Long-term reprogramming of innate immunity, that induces adaptive traits and has been termed *trained immunity* characterizes prototypical innate immune cells such as natural killer cells and monocytes, and provides protection against reinfection in a T/B-cell-independent manner. In contrast, *trained immunity* has been shown to be able to induce protection against reinfection in a lymphocyte-independent manner. Non-specific protective effects dependent on *trained immunity* have also been shown to be induced after BCG vaccination in humans. Specific signaling mechanisms including the dectin-1/Raf1 and NOD2-mediated pathways induce trained immunity, through induction of histone modifications (methylation, acetylation) and epigenetic reprogramming of monocyte function. Complex immunological and metabolic circuits link cell stimulation to a long-term epigenetic reprogramming of its function. The concept of *trained immunity* represents a paradigm change in immunity and its putative role in infection and inflammation may represent the next step in the design of future vaccines and immunotherapeutic approaches.

KL.07 Microbiota and cancer therapeutics

KL07.1
The unsuspected role of gut microbiota in cancer therapies

***L. Zitvogel**;*
INSERM, Gustave Roussy Cancer Center, University Paris Saclay, Villejuif, France.

We recently highlighted the crucial role of gut microbiota in eliciting innate and adaptive immune responses beneficial for the host in the context of effective therapies against cancer (chemotherapies, immunotherapy based on immune checkpoint blockers).
1/ *Context of cyclophosphamide (CTX):* Chemotherapeutic agents, by compromising, to some extent, the intestinal integrity, facilitate the gut permeability and selective translocation of Gram positive bacteria in secondary lymphoid organs. There, anti-commensal pathogenic TH17 T cell responses are primed, facilitating the accumulation of TH1 helper T cells in tumor beds post-chemotherapy as well as tumor regression. Importantly, the redox equilibrium of myeloid cells contained in the tumor microenvironment is also influenced by the intestinal microflora, contributing to tumor responses. Hence, the anticancer efficacy of alkylating agents is compromised in germ-free mice or animals treated with antibiotics. These findings represent a paradigm shift in our understanding of the mode of action of many compounds having an impact on the host-microbe mutualism (Viaud S, *Science* 2013). These findings have been extended to platinum salts (oxaliplatine, cis-platine) as well as to a combination of anti-IL-10R mAb+CpG for Iida et al. Science Nov 2013 (Trinchieri's group at the NIH, USA).
2/ *Context of CTLA4 blockade :* The immune checkpoint blocker (ICB) anti-CTLA4 Ab is a first-in class compound approved for reinstating cancer immunosurveillance and prolonging survival in metastatic patients. However, this clinical benefit is often associated with immune –related side effects at sites exposed to commensal flora such as the large intestine. Uncoupling efficacy from toxicity is a challenging issue for the future development of ICB. Her team showed (and submitted to Science) that the antitumor effects of CTLA4 blockade, largely dependent upon Toll like receptor (TLR)2/TLR4 receptors, markedly rely on the regulatory commensal *Bacteroides fragilis (Bf) (*in coordination with *Burkholderia cenocepacia).* Innate signaling induced by specific TLR2/TLR4 agonists failed to compensate the lack of tumoricidal activity mediated by CTLA4 blockade in germ free (GF) or antibiotics-treated mice while the IL-12-dependent cognate immunity directed against *Bf* could do so. Hence, anti-CTLA4 Ab elicited protective *Bf*-specific Th1 immune responses in specific pathogen free (SPF) mice that could be substituted, in GF animals, by oral *Bf*, purified *Bf*-associated polysaccharides or a *Bf*-specific adoptive T cell transfer, without triggering overt colitis. Ipilimumab could also restore *Bf*-specific Th1 immune responses in a fraction of advanced melanoma patients. This study unravels the key role of *B.fragilis* in the immunostimulatory effects of anti-CTLA4 Ab, opening up novel strategies to safely broaden its clinical efficacy (Vétizou et al. *Science Nov. 2015*). At the same time, Gajewski's group in Chicago showed that *Bifidobacteria* from the gut influence the tumor microenvironment in such a way that anti-PDL-1 Ab can induce a prominent anticancer immune responses (Sivan et al. Science Nov. 2015).
3/ *Setting of PD-1/PDL-1 blockade:* In September 20 2017, the demonstration of the deleterious role of antibiotics in the clinical efficacy of PD-1 blockade in lung, kidney and bladder cancer patients was brought up, highlighting the role of *Akkermansia muciniphila* as the main player in the immunomodulatory effects of pembrolizumab or nivolumab (Routy et al. Science 2017 Nov2). The mechanisms by which *A. muciniphila* restores gut dysbiosis will be discussed, involving CCR9 and IL-12.
From these findings, we infer that oncomicrobiotics and/or fecal microbial transplantation could be considered as adjuvants to the current oncological armamentarium in dysbiotic cancer bearers.

KL.08 The maintenance and mobilization of resident immune memory cells

KL08.1
The maintenance and mobilization of resident immune memory cells

***A. Radbruch**[1,2];*
[1]Deutsches Rheumaforschungszentrum, Berlin, Germany, [2]Charité University Medicine, Berlin, Germany.

Populations of memory T lymphocytes and memory plasma cells residing in epithelial tissues and in the bone marrow provide first-line protection and longterm memory to prevailing antigenic challenges of the environment. We have now also identified memory B lymphocytes of the bone marrow as a population distinct from their splenic counterparts in terms of repertoire and phenotype. Apparently the resident memory lymphocytes are not maintained by homeostatic proliferation. For memory plasma cells of the bone marrow, we could demonstrate that they are maintained individually by stromal cells. Their survival is dependent on cell contact to the stromal cell, inducing PI3K signaling, and on the cytokines April or BAFF from their environment, inducing NFkB signaling. In synergy, both signaling pathways in plasma cells upregulate expression of the vital transcription factor IRF4 and prevent caspase-induced apoptosis. Memory T and B lymphocytes of the bone are maintained individually on stromal cells as well, and sensitive to inhibition of the PI3K pathway, suggesting that stromal cells play a pivotal role for immunological memory, by inducing vital, cell-contact dependent PI3K signaling. In secondary immune reactions, resident quiescent T and B lymphocytes obviously have to be mobilized from their memory niches. We could show for resident CD4+ memory T lymphocytes that this mobilization leads (a) to the formation of "Immune clusters" in the bone marrow, resulting in amplification of the specific memory lymphocytes, and (b) to the emigration of specific resident memory T lymphocytes into the blood, and their participation in the secondary immune reaction.

SYMPOSIA

S.A2 Immune development and aging from the cradle to the grave

S.A2.01
Dissecting blood and immune cell lineages by endogenous barcoding

***H. Rodewald**, W. Pei, T. Feyerabend, K. Klapproth, K. Busch, A. Schuon, T. Benz, X. Wang, J. Rössler, T. Höfer;*
German Cancer Research Center (DKFZ), Heidelberg, Germany.

Deconvolution of cell lineage origins and relationships remains a major challenge. Once achieved, this would allow deep insights into understanding not only the formation and maintenance of tissues but also of stem and progenitor cell fates and clone sizes. Genetic fate mapping enables the tracing of the origins and lineages but numbers of available fluorescent markers have been limiting. Within the immune system, hematopoietic stem (HSC) and progenitor cells have been studied in cellular and molecular details, but information on the physiological behavior of HSC under non-perturbed or challenge conditions is limited. We have developed an endogenous genetic HSC fate mapping system that revealed differentiation rates of HSC in the bone marrow and the differentiation flow from stem cells via progenitors in vivo (Busch et al. Nature 2015; reviewed in Hoefer and Rodewald Blood 2018). To combine fate mapping with high resolution barcoding of individual cells we devised a barcoding system, in which an artificial recombination locus (termed *Polylox*) serves as Cre recombinase-dependent substrate in cells in vivo (Pei, Feyerabend et al. Nature 2017). We have introduced barcodes in embryonic HSC progenitors and in adult HSC and studied the lineage outputs from HSC clones, as well as their sizes in the bone marrow. The data demonstrate common myeloid-erythroid, and common lymphocyte pathways as fundamental structures of the hematopoietic system. These endogenous barcoding studies are now exploited to unravel at high resolution lineage relationships of tissue resident macrophages of embryonic origins.

S.A2.02
Implications from monogenic autoimmunity

***K. Kisand**;*
University of Tartu, Tartu, Estonia.

Autoimmunity caused by single gene defects is a rapidly growing group of hereditary diseases. Autoimmune tissue damage is often accompanied by susceptibility to certain infectious agents highlighting the dual function of the immune system - to recognize pathogens and secure tolerance to own tissues. APECED (autoimmune polyendocrinopathy candidiasis ectodermal dystrophy) is caused by *AIRE* gene mutations resulting in defective central T cell tolerance. However, the most striking feature of APECED patients is the presence of multiple high-titer autoantibodies that target cytokines: type I interferons and Th17 cytokines. Monoclonal auto-antibodies isolated from patients' B cells show extremely high affinity which has permitted their application for diagnostic and potentially therapeutic purposes. STAT1 gain-of-function (GOF) mutation is another gene defect that leads to chronic mucocutaneous candidiasis and variable manifestations of autoimmunity. In these patients the development of Th17 cells, that are indispensable for fungal protection, is impaired. The precise molecular mechanisms causing STAT1 signalling imbalance in STAT1 GOF cells are still unclear. We have applied ChIP-Seq to shed some light on these processes. In spite of the rarity of monogenic autoimmune diseases their impact on unfolding of the processes that shape and regulate the immune responses is immense.

S.A2.03
Strategies for enhancing human immunity during ageing

***A. N. Akbar**;*
University College London, London, United Kingdom.

Older people suffer from increased incidence and severity of infections and also cancer. In addition, vaccination therapies becomes less efficient with age. It is possible to enhance the function of immune cells during ageing in both humans and mice by blocking the function of a group of proteins called the sestrins
(Lanna et al Nature Immunology 2017). The sestrins were found to form a molecular complex, termed sMAC (sestrin-induced MAPK activation complex), with p38 and also the other two classes of MAPKs namely ERK and JNK in these cells. This for the first time provides a mechanism for integrated activation of all three classes of MAPK within a single cell type. The inhibition of sestrins in human T cells or in genetically modified mice that are sestrin-deficient, enhanced the function of T lymphocytes. Moreover, when old sestrin deficient mice were vaccinated against influenza, the response was considerably enhanced as compared to old sestrin replete animals. Therefore temporary immune enhancement by short-term inhibition of sestrins can boost immune function that could be beneficial during anti-ageing immunotherapy, for instance during vaccination of older humans.

S.A3 Immunomonitoring and biomarkers

S.A3.03
CD Maps - antigen density measurements of CD1-CD100 on human lymphocytes and thymocytes

***T. Kalina**[1], J. Stuchlý[1], D. Kužílková[1], K. Fišer[1], M. Cuenca[2], E. Blanco Álvarez[3], S. J. W. Bartol[4], N. Brdičková[1], M. Andres-Perez[3], M. C. van Zelm[5], P. Engel[2];*
[1]Charles University, Prague, Czech Republic, [2]University of Barcelona, Barcelona, Spain, [3]University of Salamanca, Salamanca, Spain, [4]Erasmus MC, Rotterdam, Netherlands, [5]Monash University, Melbourne, Australia.

Leucocyte receptors have been characterized using antibodies validated by the human leucocyte typing HLDA/HCDM organization (www.hcdm.org). However, expression information is outdated or incomplete. CD Maps project aims at quantitative mapping of expression of all CD molecules across the spectrum of leucocyte subsets. We measured PE conjugated CD1-CD100 antibodies in the context of four standardized 8-color panels on cells from three tissues (blood, thymus and tonsil). We attempted to re-map CD markers expression in thymocytes using a 37 marker mass cytometry panel (29 surface markers and 8 transcription factors). We expert gated 9 thymocyte stages based on canonical markers (CD34, CD1a, CD3, CD4, and CD8). We developed fast time and paths inference algorithm, which involves branching and also accounts for possible alternative developmental paths. We processed the thymocytes through computational algorithm in parallel to expert gating. The simulated paths reached the canonical CD4 helpers end in 79% and the canonical CD8 end in 19% respectively. The CDMaps project generates a broad and updated online database containing the expression profiles of all CD markers on human leukocyte subsets present in blood, tonsil, and thymus. Quantitative information on receptor expression is important for mechanistic studies as well as flowcytometric panel design and design of novel biological therapeuticals. Therefore it will serve as a useful resource to widen and advance studies into basic, translational and clinical immunology. CD Maps project is supported with reagents by BD Biosciences, BioLegend, Exbio and Fluidigm. TK is supported by Ministry of Health Czech Republic grant 15-26588A and LO1604.

S.A4 Germinal centers and B cell differentiation

S.A4.01
Germinal centers under the lens: Differentiation of plasma cells and memory B cells from germinal center precursors

***R. Brink**[1,2];*
[1]Garvan Institute, Darlinghurst NSW, Australia, [2]UNSW Australia, Darlinghurst NSW, Australia.

Germinal centers form in secondary lymphoid organs in response to challenge with T-cell-dependent antigens. After extrafollicular and follicular interactions with cognate antigen and T helper cells, responding B cell clones can enter the germinal center response, where they undergo iterative cycles of proliferation, somatic hypermutation and selection such that clones acquiring increased affinity for the eliciting antigen preferentially accumulate. This process is also associated with the differentiation of long-lived memory B cells and antibody-secreting plasma cells from germinal center B cell precursors. These two populations facilitate long-term immunity against infectious pathogens and underpin the efficacy of almost all current vaccines.
Although the importance of memory B cells and plasma cells to long-term immunity is well established, the processes that regulate their production during the germinal center response have been difficult to identify due to the dynamic nature of the geminal center and the difficulty of tracking the antigen specificity and fate of responding B cells. We have used a high resolution in vivo model in which B cells respond to a low affinity variant of the model antigen hen-egg lysozyme, to identify the precursors of both memory B cells and plasma cells in the germinal center. A fundamental dichotomy in the differentiation of these two critical germinal center outputs was revealed in terms of both antigen affinity and cell cycle status. This system not only optimises the specificity and potency of the antibody response but provides response flexibility within the long-term memory B cell pool.

S.A5 Initiation of immune responses

S.A5.01
Regulation of cytotoxic responses

***G. Guarda**;*
IRB, Bellinzona, Switzerland.

Natural Killer (NK) cells are cytotoxic lymphocytes that contribute to the elimination of virally infected or transformed cells. Their cytotoxic activity is regulated by two complementary sets of receptors; the activating receptors, which are engaged by stress-induced molecules, and the inhibitory ones, recognizing MHCclass I molecules.NK lymphocytes are extremely powerful and several efforts are undertaken in order to to harness their full potential for therapeutic purposes. A deeper understanding of the mechanisms regulating their development, survival, and activity, is therefore needed. Recently, the study of mechanisms specifically regulating their activation as well as their metabolism has revealed new pathways and players relevant to NK cell-mediated immunity. Advances in our understanding of such mechanisms and the consequences on innate cytotoxic immunity will be discussed.

S.A5.02
Dendritic cells in HIV-1 sensing and restriction

***T. B. H. Geijtenbeek**[1,2];*
[1]Amsterdam UMC, Amsterdam, Netherlands, [2]Amsterdam Infection & Immunity Institute, Amsterdam, Netherlands.

Sexual transmission is the primary route of infection by HIV-1 and mucosal dendritic cell (DC) subsets are amongst the first targets for HIV-1.Although DCs are paramount to the induction of antiviral immunity to HIV-1, it is becoming evident that HIV-1 subverts DCs for dissemination to T cells as well as escape from antiviral immunity. HIV-1 productively infects submucosal DCs but this does not lead to an antiviral type I Interferon (IFN) immune response as HIV-1 escapes viral sensing in DCs. We identified the dead-box helicase DDX3 as a RNA sensor for HIV-1 and our recent data strongly suggest that HIV-1 blocks this viral sensor in DCs, preventing triggering of antiviral immunity Strikingly, interfering with the inhibitory pathway, leads to efficient DC maturation, type I IFN and cytokine responses, which limits HIV-1 replication in vitro and in vivo. Here, we will discuss the importance of DDX3 in sensing HIV-1 replication and induction of innate and adaptive immunity. Furthermore, we will discuss how synthetic DDX3 ligands can be used as adjuvants in immunotherapy to induce innate and adaptive immune responses. However, not all DC subsets become infected by HIV-1 as our data show that mucosal Langerhans cells (LCs) are resistant to HIV-1 infection. LCs efficiently capture HIV-1 and route the virus into a autophagy-mediated degradation pathway, which prevents infection. Here I will discuss the molecular mechanisms underlying the distinct functions in DC subsets in HIV-1 infection and how we can harness these mechanisms to prevent infection and enhance antiviral immunity.

S.A5.03
The role of Complement in Tolerance

***M. Botto**;*
Imperial College London, London, United Kingdom.

Complement has been shown to contribute to the immunopathology of several autoimmune diseases including systemic lupus erythematosus (SLE). Paradoxically, however, complement also appears able to protect against autoimmunity since complement deficiencies, particularly C1q deficiency, strongly predispose to the development of SLE. There are currently several proposed mechanisms whereby deficiency or low levels of complement might lead to break of tolerance, and these are not mutually exclusive.One of the hypotheses to explain the heightened susceptibility to the development of SLE in the absence of C1q invokes an important role for complement in the waste-disposal mechanisms of dying cells. However, impaired clearance of such cells is, on its own, insufficient to induce autoimmunity. The data available from knockout mice emphasize that the break of tolerance depends on many factors in addition to the defective removal of dying cells. Recent findings have highlighted that C1q and C3 can modulate both adaptive and innate immune responses. In addition, C1q may not only act as initiator of the classical complement pathway, but can also mediate multiple immune responses in a complement activation independent manner. In particular, C1q can restrain autoimmunity by acting as a metabolic regulator of effector $CD8^+$ T cells. In summary,the traditional view of the role of complement in tolerance needs revision as evidence is emerging of an important interplay between complement and immunometabolism in autoimmunity

S.B1 Tumor vaccination principles and immuno therapy

S.B1.02
Next generation Immunotherapies

***E. Vivier**;*
Aix Marseille Univ, CNRS, INSERM, APHM, CIML, Innate Pharma,, Marseille, France.

Immuno-oncology, including checkpoint inhibitors targeting the PD-1/PD-L1 (PD-x) axis in particular, has revolutionized cancer treatment. However, only a subset of patients respond to these immunotherapies, and the development of drug resistance is frequent. Here, we report that the blocking of the inhibitory NKG2A receptor enhances tumor immunity by promoting both Natural Killer (NK) and $CD8^+$T-cell effector functions in mice and humans. Monalizumab, a humanized anti-NKG2A antibody, enhanced NK cell activity against various tumor cells and rescued $CD8^+$ T-cell function in combination with PD-x axis blockade. Monalizumab also stimulated NK-cell activity against antibody-coated target cells. We also established proof-of-principle for the use of combined immunotherapy with monalizumab and cetuximab in a phase II clinical trial, in which the combination showedpromise for the treatment of patients with squamous cell carcinoma of the head and neck (SCCHN). NKG2A targeting with monalizumab is thus a novel checkpoint inhibitory mechanism promoting anti-tumor immunity by enhancing the activity of both T and NK cells, which may complementthe first-generation immunotherapies against cancer.

S.B1.03
Intratumorally produced immunoglobulin repertoires

***D. M. Chudakov**[1,2,3];*
[1]Institute of Bioorganic Chemistry, Moscow, Russian Federation, [2]Privolzhsky Research Medical University, Nizhny Novgorod, Russian Federation, [3]Central European Institute of Technology, Brno, Czech Republic.

The emerging data shows that B cells may play an essential role in the immune response to cancer - as antigen-presenting cells, and by cytokines and antibodies production. Been present at limited counts in a tumor infiltrate, B cells may participate in generation of tertiary lymphoid structures, convert to the plasma cell phenotype and produce huge amounts of antibodies, which specificity and isotype-determined functional activity may influence on either cancer surveillance or immunosuppression. From this point, repertoire of intratumorally produced antibodies represents yet poorly explored component of cancer-immunity interaction that could play its role as a biomarker or as a source of tumor-specific receptors for precise immunotherapy. I will briefly summarize current knowledge on tumor-infiltrating B cells and immunoglobulin repertoire they produce, complementing it with our recent experience on antibody repertoire profiling with high-throughput sequencing. In particular, I will cover full-length nearly error-free immunoglobulin repertoire profiling using 5'RACE with UMI, extraction of immunoglobulin repertoires from RNA-Seq data with MiXCR, and provide guidelines for repertoire diversity/clonality analysis.
Research supported by grant of the Ministry of Education and Science of the Russian Federation #14.W03.31.0005.

S.B2 Environmental regulation anti-tumor responses

S.B2.03
Dissecting immune complexity of metastatic breast cancer

***K. E. de Visser**;*
the Netherlands Cancer Institute, Oncode Institute, Amsterdam, Netherlands.

Metastasis formation is a key challenge in cancer patient care that urgently needs solutions. It is now well established that cells and mediators of the immune system influence these processes. Historically, our immune system has been considered to form an intrinsic defense mechanism against cancer and metastasis. Yet, the majority of cancer types exploit a myriad of strategies to successfully evade destruction by the immune system. In fact, mounting evidence supports the notion that cancer cells hijack the immune system for their own benefit, allowing them to escape from immune attack, maintain limitless proliferation, survive under dire circumstances and spread to distant organs. The overall goal of our research is to understand how the immune system influences metastasis formation. To achieve this, we utilize pre-clinical mouse models that faithfully recapitulate human breast tumorigenesis in combination with immune profiling studies in breast cancer patients. We have previously discovered that breast tumors elicit a systemic inflammatory cascade to dampen anti-tumor T cells and promote metastasis formation. Current efforts are underway to dissect how the genetic make-up of breast tumors dictates activation of systemic immunosuppressive inflammation. Our findings provide novel mechanistic insights into the thus far poorly understood metastatic cascade, and open new avenues for the development of therapeutic strategies to unleash anti-tumor immunity and to inhibit metastatic disease.

S.B3 The Yin and Yang of T-cell regulation

S.B3.03
Maintaining T cell tolerance via the CTLA-4 pathway

***L. S. K. Walker**;*
University College London, London, United Kingdom.

The immune system provides vital protection from infection and cancer but needs to be tightly regulated to prevent the development of autoimmune diseases such as type 1 diabetes and rheumatoid arthritis. The ability to augment or diminish the immune response in a controlled fashion holds the promise of boosting anti-tumour responses or silencing autoimmune diseases respectively. A major checkpoint controlling immune responses involves the T cell molecule CTLA-4 that is expressed at high levels in FoxP3+ regulatory T cells. CTLA-4 controls engagement of the T cell costimulatory receptor, CD28, by binding to their shared ligands and removing them from antigen presenting cells by a process of trans-endocytosis. Our lab seeks to understand how this mechanism operates in the steady state to maintain tolerance and allow appropriate immune responses to develop in a timely manner. Understanding the molecular basis of the CTLA4 checkpoint will ultimately empower us to manipulate the immune response in a more precise manner.

S.B4 T cell activation and exhaustion

S.B4.02
Immunodeficiencies associated with increased T cell senescence

***S. Kracker**, L. Heurtier, M. Deau, L. Chentout, A. Bouafia, A. Durandy, M. Cavazzana;*
INSERM UMR 1163, Imagine Institute, Paris, France.

Activated PI3Kδ syndromes 1 and 2 (APDS1 and 2) are primary immunodeficiencies caused by either gain-of-function mutations in the *PIK3CD* gene encoding p110δ (the catalytic subunit of PI3Kδ) or heterozygous mutations in the *PIK3R1* gene encoding p85α, p55α and p50α (regulatory subunits of PI3Kδ). The disease causing mutations lead to hyper-activated PI3Kδ -signalling in lymphocytes. APDS1 and 2 are combined immunodeficiencies with variable clinical phenotypes. The clinical symptoms include recurrent respiratory infections, bronchiectasis, lymphoproliferation and hypogammaglobulinemia frequently associated with elevated IgM serum level. Both diseases appear to predispose to B cell lymphomagenesis especially diffuse large B cell lymphoma and Hodgkin lymphoma. Abnormalities of B lymphocyte subpopulations, e.g. B cell lymphopenia and higher frequency of transitional B cells, and of T cell subpopulation, e.g. decreased number of naive CD4 and CD8 T cells and increased frequency of effector/effector memory CD8 T cells and CD8CD57 positive (senescent) T cells, are frequently observed in both diseases. Immunoglobulin replacement therapy, rapamycin, allogeneic hematopoietic stem cell transplantation and selective PI3Kδ-specific inhibitors (currently on clinical trials) are possible treatment options. The work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM), the Agence National de la Recherche as part of the "Investment for the Future" program: ANR-10-IAHU-01 and by ANR-15-CE15-0020 (ANR-PIKimmun), the Ligue Contre le Cancer - Comité de Paris, the Fondation ARC pour la recherche sur le Cancer, and the Centre de Référence Déficits Immunitaires Héréditaires (CEREDIH).

S.B4.03
Maintenance of "exhausted" T cells in chronic infection

***D. Zehn**;*
Munich, Germany.

Failure to clear an infection can coincide with the appearance of T cells which express low amounts of cytokines and high levels of inhibitory receptors (PD-1, Lag-3, Tim-3). This phenotype has so far been viewed to mark exhausted and terminally differentiated effector cells. Nonetheless, "exhausted" T cell populations in chronic infections and tumors are long-living and can be expanded upon blocking inhibitory receptor ligand systems. This raised the questions how these populations are maintained and which mechanisms act in case of therapeutically induced re-expansion. We and others identified recently that both long-term T cell maintenance in chronic infections and re-expansion following blockade of PD-1 signaling are depending on a small subpopulation of T cells, which express the transcription factor Tcf-1. We showed that Tcf-1 positive T cells share key features with conventional memory T cells while they co-express markers of "exhausted T cells" (i.e. PD-1). These memory-like T cells are capable of undergoing self-renewal while they are continuously generating terminally differentiated effector cells. Given the presumed central role of the memory-like population for therapeutic purposes, we will report recent advances on molecules and mechanisms that control size and function of this subset.

S.C1 Maintenance and local regulation of tissue specific immunity

S.C1.02
Functions of Resident Memory T Cells

***D. Masopust**;*
University of Minnesota, Minneapolis, MN, United States.

Resident memory T cells, often abbreviated T_{RM}, occupy tissues without recirculating and provide a first response to infections reencountered at body surfaces, where they accelerate pathogen clearance. T_{RM} also likely play critical roles in tumor immunosurveillance, and may contribute to immune disorders, allergies, and autoimmunity. This talk will share recent and ongoing investigations of T_{RM} function. Evidence will be presented that T_{RM} are capable of autonomously regulating the expansion of local immunosurveillance independently of central memory or proliferation in lymphoid tissue. Data will be communicated that reveal a nonlymphoid origin of secondary lymphoid organ T_{RM} and that suggest vaccination strategies by which memory CD8 T cell immunosurveillance can be regionalized to specific lymph nodes. Experiments supporting developmental plasticity among T_{RM} will be reviewed. Lastly, evidence will be presented that natural antiviral T_{RM} can be repurposed to control or eliminate tumors.

S.C1.03
Activation of Ca2+ regulated pathways downstream of Pattern Recognition Receptors

***F. Granucci**[1,2], L. Marongiu[2], L. Gornati[2], I. Artuso[2], F. Mingozzi[2], I. Zanoni[2];*
[1]Milan, Italy, [2]University of Milano-Bicocca, Milan, Italy.

Microorganism invasions are perceived by pattern recognition receptor (PRR)-expressing cells of the innate immune system. Among PRR, TLRs and their co-receptors are the best characterized. CD14, with LPS binding protein (LBP), TLR4 and MD-2 forms the multi-receptor complex for LPS. CD14 is a glycosylphosphatidylinisitol (GPI)-anchored protein abundantly expressed on DCs and macrophages. CD14 concentrates the LPS signal, mediates the relocation of TLR4 and MD2 to the endosome for the initiation of the TRIF signaling pathway and is responsible for Ca^{2+} mobilization and NFAT signaling pathway activation in DCs. Ca^{2+} mobilization is one of the first events for the initiation of the NFAT signaling pathway. We have investigated the mechanism of Ca^{2+} mobilization leading to NFAT activation in myeloid mouse and human DCs following LPS stimulation. Following LPS stimulation, IP_3 second messenger induces a SOCE through IP_3 receptor 3 channels co-localized with CD14. For this process to occur, Ins(1,3,4,5)P_4 generation by the IP_3 kinase (ITPK) B is required to antagonize IP_3 dephosphorylation and increase IP_3 availability. ITPKs pharmacological inhibition restrains inflammatory events (such as increased vessel permeability or inflammatory arthritis) regulated by NFATs in the presence of LPS, similar to the direct inhibition of NFATs by nanodrugs. ITPKB represents a new target for anti-inflammatory therapies aimed at inhibiting specific DC functions. The NFAT-controlled phenomena and the consequences of NFAT activation in innate immune cells will be discussed in models of microbial infections and sterile inflammation.

S.C2 Immune signalling and therapy in autoimmunity

S.C2.01
Myeloid and glial cells collaborate to regulate neuroinflammation

***T. Owens**[1], G. Webster[2], R. Khorooshi[1], J. Marczynska[1], A. Wlodarczyk[1], A. Benmamar-Badel[1], R. Dieu[1];*
[1]University of Southern Denmark, Odense, Denmark, [2]Innate Immunotherapeutics, Auckland, New Zealand.

Tissue macrophages and blood-derived regulatory myeloid cells play important roles in development and normal homeostasis. In the neonatal central nervous system (CNS) a subset of tissue-resident $CD45^{low}CD11b^{+}$ microglia that express CD11c dramatically expand, and are a critical source of myelinogenic IGF1. Transfer of such cells to adult mice alleviated experimental autoimmune encephalomyelitis (EAE) which identifies an anti-inflammatory role for this microglial subset in the developing CNS, whose mechanism remains to be established. We have also examined how innate signaling can direct anti-inflammatory myeloid cell programs in the adult CNS. Microglia as well as extraparenchymal $CD45^{high}CD11b^{+}$ macrophages were induced to produce interferon-alpha and -beta by intrathecal TLR3/RIG-I ligand poly-I:C, and this alleviated EAE. Intrathecal administration of a bi-specific NOD2 and TLR9 microparticle (MIS416) induced marked influx from blood of $CD45^{high}F4/80^{+}GR\text{-}1^{-}CD11b^{+}CD11c^{+}$ monocytes as well as $CD45^{high}Gr\text{-}1^{high}Ly6G^{high}CD11b^{+}CD11c^{-}$ neutrophils, as early as 2h after injection.

Both populations phagocytosed MIS416, produced IFNβετα and expressed PDL1. Intrathecal MIS416 alleviated EAE and this was dependent on IFNAR signaling. RNAseq analysis showed upregulation in the CNS of NFκB, Jak-STAT, TLR and NLR signaling pathways, as well as IFNgamma and neutrophil- (CXCL1, CXCL2) and monocyte-attracting (CCL2, CXCL10) chemokines. Microglia were a prominent source of chemokines. These studies collectively show that CNS-intrinsic signals promote and recruit endogenous and exogenous myeloid programs that collaborate to maintain CNS homeostasis in development, and in response to infection or tissue damage.

S.C2.03
How to modulate TCR signalling for peptide therapy in autoimmunity

***D. C. Wraith**;*
University of Birmingham, Birmingham, United Kingdom.

Control of autoimmune and allergic conditions can be reinforced by tolerance induction with peptide epitopes; this presentation will focus on the mechanisms involved. Peptides must mimic naturally processed epitopes and be designed to target tolerogenic, steady-state dendritic cells (DCs). Steady-state DCs express low levels of costimulatory molecules and induce 'abortive' activation of T cells whereby T cells undergo initial cell division but do not differentiate. $CD4^+$ T-cells become anergic following their first encounter with peptide. This depends on strength of signal via the T cell receptor (TCR) and the balance between TCR and costimulatory signalling. Continuation of peptide therapy results in the generation of anergic, IL-10 secreting $CD4^+$ T-cells with regulatory function. The loss of proliferative capacity correlates with a cytokine switch from a pro-inflammatory to a phenotype characterised by secretion of the anti-inflammatory cytokine IL-10. The IL-10 secreting Tr1-like cells suppress dendritic cell maturation, prevent Th cell differentiation and create a negative feedback loop for Th driven immune pathology. This mechanism leads to bystander suppression whereby Tr1-like cells specific for one antigen can suppress T cells specific for other antigens derived from the same tissue. Tolerance induction involves upregulation of transcription factors controlling IL-10 and inhibitory receptors limiting T cell signalling. The peptide therapy approach is a highly selective approach for prevention and treatment of autoimmune diseases in humans. Results from clinical trials of peptide immunotherapy in multiple sclerosis and Graves' disease will be described.

S.C3 Transplantation

S.C3.01
Treating autoimmune diseases with T regulatory cells - first clinical data

***P. Trzonkowski**;*
Medical University of Gdansk, Gdansk, Poland.

T regulatory cells (Tregs) are considered a viable option in tolerance induction treatment in the clinic. First promising clinical experiments and trials with clinical-grade Tregs cultured as advanced therapy medicinal product (ATMP) are completed already. We will present long-term results (up to 5 years follow up) as well as our ongoing trials with Tregs in type 1 diabetes and multiple sclerosis discussing metabolic and immune background of the patients, which, in our opinion, influenced the efficacy of this treatment. In vivo results will be supported with in vitro and animal models showing activity of Tregs in auto- and allogeneic settings. References: J Transl Med. 2016;14(1):332, Diabetes Care. 2012;35(9):1817-20; Ann Surg. 2011;254(3):512-8; Clin Immunol. 2009;133(1):22-6

S.C3.03
Transplantation, T cell engenering

***C. Bonini**;*
UNIVERSITA' VITA SALUTE SAN RAFFAELE, Milan, Italy.

Transplantation, T cell engineering
Chiara Bonini, Università Vita-Salute San Raffaele
Ospedale San Raffaele Scientific Institute
Milano
Adoptive T cell therapy represents an innovative and promising therapeutic approach, which relies on the ability of T lymphocytes to recognize and destroy specific targets on microbes or tumors through their T cell receptors (TCR), to obtain efficient killing of cancer cells. Ideally, adoptively transferred T cells should be: 1. Specific for tumor antigens, 2. Able to expand and persist long enough to mediate a long lasting clinical response, 3. Able to counteract the immunosuppressive tumor microenvironment.
TCR genetic engineering represents a suitable approach to generate large numbers of tumor specific T cells. The core of this approach is the transfer in patients' T cells of genes encoding for rare tumor-specific TCR. The simple transfer of tumor specific TCR genes into T cells is affected by some limitations: genetically modified T cells shall express four different TCR chains, that might mispair, leading to unpredictable toxicity and to an overall dilution of the tumor specific TCR on lymphocyte surface, thus limiting the efficacy of therapeutic cellular product. To overcome these issues, we can adopt a TCR gene editing approach, based on the concomitant disruption of the endogenous TCR genes and introduction of the tumor specific TCR genes. Different protocols to generate high numbers of TCR edited memory stem T cells and central memory T cells, able to overcome the immunosuppressive tumor microenvironment will be discussed.

S.C4 Manipulation of tolerance

S.C4.01
Engineered Dendritic Cells to re-establish Antigen-Specific Tolerance in T-cell Mediated Diseases

***S. Gregori**;*
San Raffaele Telethon Institute for Gene Therapy (SR-TIGET), Milan, Italy.

The design of novel approaches to control antigen (Ag)-specific pathogenic T cell responses and restore tolerance represents an ambitious goal for the management of autoimmune diseases. The prominent role of dendritic cells (DC) in promoting T-cell tolerance and the development of methods to generate clinical grade products allowed the clinical application of tolerogenic DC-based therapies for the control of unwanted immune responses. The concluded clinical trials demonstrated the safety and feasibility of this approach. However, the stability of the infused DC products and the maintenance of their tolerogenic properties *in vivo* remain open issues to be tackled for improving the safety and the efficacy of DC-based cell therapies. Our hypothesis is thatinfusion oftolerogenic DC genetically modified by newly developed tolerogenic lentiviral vectors (LV) encoding autoAg-derived epitopes (tolLV-DC) will promote the *in vivo*generation of Ag-specific tolerance *via*down-regulation of autoAg-specific pathogenic T cell responses and induction of long-living autoAg-specific Tregs. To this aim we developedLV-platforms that allow the expression of specific autoAg epitope and pro-tolerogenic molecules. Our preliminary data show that tolLV-DC can modulate T cell responses both *in vitro*and *in vivo*and that infusion of tolLV-DC dampens Ag-specific T cell responses *in vivo*. The success of our strategies will help designing a safer tolDC-based cell therapy, abrogating the boosting of autoimmunity, and to stably preserve the tolerogenic properties of *in vivo* transferred DC.

S.C4.02
The role of cell metabolism in Treg biology and function

***B. Salomon**, R. Vallion, J. Divoux, S. Gregoire, E. Ronin;*
CIMI-Paris, Sorbonne Université, Inserm, CNRS, Paris, France.

Foxp3 regulatory T cells (Treg) play a major role in regulation of immune responses. Cellular metabolism of conventional T cells (Tconv) has been intensely studied revealing, among other findings, that their activation requires a switch from AMPK to mTOR driven metabolic pathways. We know little about cell metabolism on Treg homeostasis and function and published data are quite controversial. Thus, we have investigated the role of AMPK (coded by *Prkaa*) and mTOR in Treg by generating mice that have a conditional knockout of these molecules specifically in Treg. Whereas the *Foxp3^{cre} x Prkaa1^{lox}* (AMPKΔTreg) mice looked healthy, the *Foxp3^{cre} x mTor^{lox}* (mTORΔTreg) mice developed a systemic inflammatory disorder with massive immune cell infiltration, activation of Tconv, increased levels of inflammatory cytokines and immunoglobulins and died by 10 weeks of age. Interestingly, mTORΔTreg mice had increased Treg frequency in lymphoid organs but decreased Treg frequency in non-lymphoid organs, which was correlated with lower proliferation, migration and stability of mTOR-deficient Treg. In the healthy AMPKΔTreg mice, AMPK deficient Treg had decreased OXPHOS activity. In a cancer model, tumor growth was reduced in these mice, which was correlated with decreased proportion of tumor-infiltrating Treg and higher activation of Tconv. Thus, AMPK seems critical for the homeostasis of Treg infiltrating tumors but dispensable at steady state. Our results reveal a new role of the AMPK/mTOR metabolic balance in Treg biology.

S.C4.03

Tolerogenic dendritic cells as a therapeutic strategy to induce tolerance in multiple sclerosis

***E. Martinez-Caceres**;*
Germans Trias i Pujol Hospital. Universitat Autònoma Barcelona., Badalona (Barcelona), Spain.

Multiple sclerosis (MS) is a chronic, inflammatory, and neurodegenerative disease of the central nervous system. Its prevalence is increasing worldwide, and is now considered, after traumatisms, the main cause of disability in young people. Current treatments in MS do reduce disease activity, do not decrease long-term disability and progression and must be administered lifelong, causing relevant side effects. Therefore, safer and more effective treatments are needed. In this context, a promising strategy for the attenuation of pathogenic T cells is an autologous therapy with tolerogenic dendritic cells (tolDC). Our group has developed an antigen-specific cell therapy based on autologous monocyte-derived tolDC differentiated in the presence of vitamin D3 (VitD3), loaded with a group of myelin peptides (vitD3-tolDC), to induce tolerance in MS patients. *In vitro* studies in co-culture experiments demonstrated a potent immune-regulatory activity of vitD3-tolDC, reducing lymphocyte proliferation and IFN-γ production and producing low levels of IL-10. Moreover, *in vivo* studies in the animal model of MS - experimental autoimmune encephalomyelitis - revealed a beneficial effect of vitD3-tolDC, ameliorating the severity of the disease. These pre-clinical results, as well as, reported outcomes from previous clinical trials using tolDC, have led to a Phase I/IIa clinical trial in patients with active MS as approved by the Spanish regulatory Agency (AEMPS) which is currently ongoing (Nº EudraCT: 2015-003541-26, available at ClinicalTrials.gov Identifier: NCT02903537, Tolervit-MS).

S.C5 Allergy, asthma and therapy

S.C5.01

Spontaneous protein crystallization in asthma : a new pathway for intervention

***B. Lambrecht**[1], E. Persson[2];*
[1]VIB Inflammation Research Center, Ghent, Belgium, [2]VIB, Ghent, Belgium.

Asthma is a chronic inflammatory airway disease rich in eosinophils. As early as 1853, Charcot and von Leyden described extracellular deposits of morphologically diverse crystals in airways of asthmatics. Charcot-Leyden crystals (CLC) are made from Galectin-10 (Gal10), one of the most abundant yet least understood proteins in eosinophils. A pathogenic role for CLC or Gal10 in airway inflammation has not been established. Here, we show that the *ex vivo* crystal packing interface of CLC obtained from the upper airways of patients is identical to recombinant Gal10 crystals, and could be mutated to prevent auto-crystallization. Only in the crystalline state could inhaled Gal10 induce airway inflammation. Remarkably, Gal10 crystals when co-administered with harmless antigens could stimulate humoral and cellular immunity, promoting Th2 sensitization and airway eosinophilia *in vivo* in a mouse model. To target this type of crystal-induced inflammation in humans, we generated llama antibodies against crystalline Gal10 that rapidly dissolved preformed Gal10 crystals and CLC in the mucus of patients. In a humanized severe combined immunodeficiency model of allergic airway inflammation, administration of crystal dissolving antibodies suppressed lung inflammation, human IgE synthesis and airway mucin production. As a mechanism of action, the crystal dissolving antibodies targeted the key crystal packing contacts underlying the intrinsic auto-crystallization behavior of Gal10. Together, our data demonstrate that beyond serving as markers of eosinophilia, CLC actively promote inflammation and adaptive immunity. As protein crystallization is reversible by antibody treatment, antagonizing the crystalline state of pro-inflammatory proteins in a new intervention strategy.

S.C5.02

Isotype-specific regulation of BCR signal transduction

***J. Wienands**, N. Engels, M. Engelke;*
Institute for Cellular & Molecular Immunology, University Medical Center Göttingen, Göttingen, Germany.

The key role of the B cell antigen receptor (BCR) in triggering antibody-mediated immune responses is well established. However, it has become clear more recently that BCR ligation does not deliver an all-or-nothing signal for B cell activation. Multiple levels of BCR-intrinsic fine tuning exist that can amplify or attenuate the primary 'Go Signal' provided by phosphorylated Immunoreceptor Tyrosine-based Activation Motifs (ITAMs) and the associated kinase Syk. While ITAMs reside in the cytoplasmic segments of the canonical BCR signal elements, Ig-α and Ig-β, a second phosphorylation module is accommodated in membrane-bound IgG and IgE on class-switched memory B cells. It is called the Ig Tail Tyrosine (ITT) motif and recruits the versatile signaling protein Grb2. The phospho-ITT/Grb2 axis provides a co-stimulatory signal that renders antigen-experienced B cells less dependent on T cell help during recall responses. Indeed, memory B cell responses are dominated by class-switched Ig isotypes, most notably IgG. Given the anaphylactic properties of IgE antibodies, activation of IgE-positive B cells is tightly regulated. The long isoform of membrane-bound IgE on human B cells encompasses an ER retention motif in its extracellular membrane-proximal domain that restricts surface IgE-BCR expression and concomitant downstream signaling events. BCR-autonomous signal inhibition can also be brought about by linking up with negative regulatory modules such as lipid phosphatase SHIP known to limit BCR activation through inhibitory coreceptors. In summary, the BCR can regulate its signal output by various mechanisms to promote protective humoral immunity or keep potentially harmful B cells in check.

S.C5.03

Genetic restriction of antigen-presentation dictates allergic sensitization and disease in humanized mice

*A. Neunkirchner[1], B. Kratzer[1], C. Köhler[1], U. Smole[1], L. Mager[1], K. Schmetterer[1], D. Trapin[1], V. Leb-Reichl[1], E. Rosloniec[2], R. Naumann[3], L. Kenner[1], B. Jahn-Schmid[1], B. Bohle[1], R. Valenta[1], **W. Pickl**[1];*
[1]Medical University of Vienna, Vienna, Austria, [2]Memphis Vetrans Affairs Medical Center, Mamphis, TN, United States, [3]Max Planck Inst Molecular Cell Biology Genetics, Dresden, Germany.

IgE-associated allergies result from misguided immune responses against usually innocuous environmental or food antigens. $CD4^+$ T lymphocytes are critical for initiating and perpetuating that process, yet the crucial factors determining whether an individual gets allergic are largely unknown. We here created a novel human TCR and HLA-DR1 (TCR/DR1) transgenic mouse model of asthma, which specifically reacts to the human-relevant major pollen allergen from *Artemisia vulgaris* (mugwort) to examine these critical factors upon natural allergen exposure via the airways in the absence of systemic priming and adjuvants. We discovered that acute allergen exposure led to IgE-independent airway hyperreactivity (AHR) and Th2-prone lung inflammation in TCR/DR1, but not DR1, TCR or WT control mice, that was alleviated by prophylactic IL-2/αIL-2 mAb complex-induced expansion of Tregs. In contrast, chronic allergen exposure sensitized one third of single DR1 transgenic mice, however, without impacting on lung function. Similar treatment led to AHR and Th2-driven lung pathology in >90% of TCR/DR1 mice. Prophylactic and therapeutic expansion of Tregs with IL-2/αIL-2 mAb complexes blocked the generation and boosting of allergen-specific IgE associated with chronic allergen exposure. Our findings reveal allergen-specific T effector and Treg cell frequencies next to genetic restriction of allergen-presentation, as important factors for allergy.

S.C6 Innate control of inflammation and tissue repair

S.C6.01

The yin yang of phagocyte regulation and inflammatory cytokines

***A. Mantovani**;*
Istituto Clinico Humanitas, Humanitas University, Rozzano, Italy.

The tumor microenvironment (TME) is a complex network, which includes soluble factors and components of the extracellular matrix as well as stromal, endothelial and immune cells. Immune cells and, among them, myeloid cells, play important roles in cancer development and can promote or inhibit cancer initiation and progression. Among tumor-infiltrating immune cells, macrophages are well-known determinants of cancer-related inflammation and are typically characterized by their remarkable plasticity. This consists in the ability to acquire a wide spectrum of activation states in response to various signals derived from the microenvironment. Classical M1 and alternative M2 macrophages represent the paradigm of this property. Tumor-associated macrophages (TAMs) usually display a so-called "M2-like" phenotype that can foster tumor progression in different ways, namely by promoting genetic instability, angiogenesis and metastasis and by restraining anti-tumor adaptive immunity. Notably, TAMs can also play a dual role in the response to conventional anti-tumor therapies: they can enhance the anti-neoplastic effect or, in contrast, they can sustain a tumor-promoting response and so foil the anti-cancer power of these drugs. We recently identified IL-1R8, which we had cloned as TIR8 and is also known a SIGIRR, as a checkpoint in NK cells, which negatively regulates response to myeloid derived IL-18. Unleashed NK cells mediate resistance to liver carcinogenesis and metastasis at NK rich anatomical sites. Thus the organ immunological context is a key determinant of the role of innate and adaptive immunity in tumor progression.

S.C6.02
Extracellular DNA traps: neutrophils, eosinophils, basophils

***H. Simon**;*
Institute of Pharmacology, Bern, Switzerland.

Extracellular DNA traps represent an important element of the innate immune response and they are seen in association with many infectious, allergic, and autoimmune diseases. They are able to kill bacteria and can be formed by neutrophils, eosinophils and basophils. Although the functional importance of extracellular DNA traps is generally accepted, the origin of the DNA scaffold, as well as the mechanism of their generation, remains unclear and a matter of dispute. In my presentation, I will focus on the role of Optic Atrophy 1 (OPA1), a mitochondrial inner membrane protein known for its role in mitochondrial fusion and structural integrity. Dysfunctional OPA1 mutations cause atrophy of the optic nerve leading to blindness. We demonstrate that lack of OPA1 reduces the activity of mitochondrial electron transport complex I in neutrophils, which, owing to lowered NAD^+ availability, causes a decline in adenosine-triphosphate (ATP) production through glycolysis. OPA1-dependent ATP production in these cells is required for microtubule network assembly and for the formation of neutrophil extracellular traps (NETs). Moreover, conditional knockout mice lacking *Opa1* in neutrophils ($Opa1^{N\Delta}$) exhibit a reduced antibacterial defense capability against *Pseudomonas aeruginosa*. Hence, these findings establish an impact of OPA1 function on the innate immune system.

S.C6.03
Mitochondrial failure in monocytes immunocompromise the NLRP3 inflammasome in human sepsis

***P. Pelegrin**;*
Biomedical Research Institute of Murcia-Hospital Virgen de la Arrixaca, Murcia, Spain.

Sepsis is the leading cause of death in critical-care units. Systemic infection in sepsis induces the release of pro-inflammatory cytokines by monocytes, causing an uncontrolled inflammatory response damaging different tissues and organs. This inflammatory response is followed by an acute immunoparalysis due to broad defects in monocytes metabolism after the infection. In this study, we aim to characterize potential mechanisms leading to mitochondrial metabolism downregulation in monocytes and its implication in the immunoparalysis response of septic patients. We analyzed monocytes from patients with abdominal origin sepsis compared with control groups of healthy donors and patients undergoing abdominal surgery but not developing sepsis. We found that the cell surface expression of the ion channel P2X7 receptor increased in septic monocytes when compared with the control groups. Despite the increase of P2X7 receptor, in septic patients ATP failed to induce NLRP3 inflammasome activation in monocytes measured by ASC aggregation and IL-1beta release. In septic patients, P2X7 receptor expression in monocytes correlated with a lack of mitochondrial membrane potential. P2X7 receptor stimulation in human monocytes from healthy individuals before LPS-priming, induced a decrease of mitochondrial membrane potential and impaired the respond of the monocytes to LPS and the engagement of the NLRP3 inflammasome. Our results suggest that during sepsis P2X7 receptor expression increases in monocytes and damage mitochondria contributing to the immunoparalysis of these patients.

S.D2 Innate lymphoid cells - a topic of debate

S.D2.01
Using super-resolution microscopy to watch immune cells kill

***D. M. Davis**;*
University of Manchester, Manchester, United Kingdom.

Natural Killer (NK) cells can directly kill diseased cells by secretion of cytolytic granules across an immune synapse. The molecular choreography that leads to assembly of the synapse and the secretion of granules has widely been studied. However, a long-standing gap in our understanding of this process is how disassembly of the synapse occurs, allowing NK cells to dissociate from target cells. Using microscopy to visualize degranulation from individual NK cells, we found that the outcome of sequential stimulation depended upon the order in which different NK receptors were ligated. Moreover, we found that shedding of the Fc receptor CD16 increased NK cell motility and facilitated detachment of NK cells from opsonized target cells. Disassembly of the immune synapse caused by CD16 shedding aided NK cell survival and boosted serial engagement of target cells. Thus, counter-intuitively, shedding of CD16 can positively impact immune responses. In a separate line of research, using super-resolution microscopy, we have found that inhibitory Killer Ig-like receptors (KIR) encoded by different genes and alleles organise differently at the surface of primary human NK cells. KIR which are expressed at a low level at the cell surface assemble in smaller clusters than KIR which are highly expressed. Upon receptor triggering, lowly expressed receptors generate more phosphorylated Crk than highly expressed receptors. Thus, genetic variation modulates the nanoscale organisation of inhibitory KIR, which in turn impacts receptor signalling. This identifies a new way in which genetic diversity could impact immune responses.

S.D3 Novel approaches to vaccinology

S.D3.01
Viral vaccine delivery systems

***S. Gilbert**;*
Jenner Institute, University of Oxford, Oxford, United Kingdom.

Viral vectored vaccines are in development for many different vaccines to prevent infections, and also as therapeutic vaccines against cancer. As platform technologies they have many advantages, including reduced development timelines, ease of manufacture and the ability to employ thermostable formulations. This talk will include some recent developments in the use of adenoviral vectored vaccines against emerging pathogens, covering both preclinical and clinical data. The Jenner Institute has established a pipeline for early stage vaccine development with all activities from vaccine design, pre-clinical production and testing, GMP manufacturing and phase I clinical trials all taking place on the same campus in Oxford.
This approach has facilitated the early development of multiple novel vaccines.

S.D3.03
Therapeutic monoclonal antibodies

***A. Lanzavecchia**;*
Institute for Research in Biomedicine, Bellinzona, Switzerland.

We use cell culture-based high-throughput screens to isolate monoclonal antibodies selected for neutralizing potency and breadth. Recently, we focused on the antibody response to *P. falciparum*. In one study (Pieper et al, *Nature* 2017), we discovered that up to 10% of malaria-infected individuals produce a new type of antibodies that contain templated DNA insertions encoding the extracellular domain of LAIR1, a collagen binding inhibitory receptor encoded on chr19. These insertions are found either at the V-DJ junction or in the switch region, leading to the positioning of the LAIR1 domain on the tip of HCDR3 or in the VH-CH1 elbow. The inserted LAIR1 domain is necessary and sufficient for binding to infected erythrocytes and somatic mutations abolish collagen binding and modulate binding activity to the parasite antigens, which we identified as distinct RIFINs. Templated insertions are frequently found in memory B cells of healthy individuals, suggesting that this represents a new mechanism of antibody diversification. In another study (Tan et al *Nat Med* 2018), we analysed the antibody response of African individuals immunized by repeated injection of irradiated sporozoites. All antibodies isolated bound to the circumsporozoite protein (CSP) and recognized distinct epitopes. Strikingly, the most effective antibodies bound not only to the NANP-repeat region, but also to an N-terminal NPDP peptide that is not present in the RTS,S vaccine. These dual-specific antibodies were isolated from different donors and were encoded by certain VH3-30 alleles and provide relevant information for lineage-targeted vaccine design and passive immunization strategies.

S.D4 Exploiting host pathogen interaction

S.D4.01
Immune suppression by Salmonella

***D. Holden**;*
Imperial College, London, United Kingdom.

Salmonella enterica can cause persistent infections such as typhoid fever. Following bacterial entry into host cells, the pathogen replicates in a membrane-bound compartment called the *Salmonella*-containing vacuole (SCV). Bacteria respond to nutritional deprivation and the acidic pH in the vacuole lumen by activating the expression of the SPI-2 type III secretion system (T3SS). After assembly of a T3SS-linked pore in the vacuole membrane, bacteria sense the near-neutral pH of the host cell cytoplasm. This triggers the translocation of bacterial virulence proteins (effectors) into the host cell. Approximately 30 different effectors are translocated by the SPI-2 T3SS. These have been implicated in several physiological activities, including the control of SCV positioning within the host cell, maintenance of vacuole membrane integrity, bacterial replication, interference with lysosome function and innate immune signaling. I will discuss our recent progress on the characterization of a unique effector that suppresses the development of adaptive immunity by inhibiting antigen presentation by MHC class II molecules. This effector is likely to contribute to the ability of *Salmonella* to persist in mammalian tissues.

S.E1 Visualizing immune responses

S.E1.02
Neuronal regulation of innate lymphoid cells

***H. Veiga-Fernandez**;*
Champalimaud Centre for the Unknown, Lisboa, Portugal.

Innate lymphoid cells (ILC) are the most recently defined cell family to be included to the increasingly complex atlas of the immune system. ILC have a lymphoid morphology, lack rearranged antigen receptors and are abundantly present at mucosal surfaces. The combined expression of lineage-specific transcription factors with discrete cytokine profiles led to the identification of distinct ILC subsets. ILC development and function have been widely perceived to be programmed. However, emerging evidence indicates that ILC are also controlled by complex environmental signals. Here, we will discuss how ILC perceive, integrate and respond to their environment, notably to nutritional and neuronal cues.

S.E1.03
Spatiotemporal dynamics of CD8$^+$ T cells undergoing intrahepatic priming

***M. Iannacone**;*
San Raffaele Scientific Institute, Milan, Italy.

CD8$^+$ T cell responses to hepatotropic intracellular pathogens such as hepatitis B virus (HBV) range from tolerance to full differentiation into effector cells endowed with antiviral potential. However, the molecular and cellular mechanisms underlying these distinct outcomes are incompletely understood. Here, we used multiphoton intravital microscopy, RNA-seq and ATAC-seq to interrogate the motility, transcriptional and epigenetic changes of naïve HBV-specific CD8$^+$ T cells undergoing intrahepatic priming. We found that intrahepatic priming can lead to both effective or dysfunctional CD8$^+$ T cell responses. Priming by Kupffer cells leads to differentiation into effector cells that are indistinguishable from those primed in secondary lymphoid organs; these effector cells form dense, poorly perfusable clusters that are scattered throughout the liver and are composed by largely immotile cells. By contrast, priming by hepatocytes leads to local activation, vigorous proliferation but lack of differentiation into inflammatory cytokine-producing and cytolytic effector cells; these dysfunctional cells accumulate in looser, intravascular clusters that coalesce around portal tracts and are composed by more motile cells. Transcriptome and epigenome analyses of these dysfunctional cells reveal a signature that is distinct from that of exhausted cells, including the lack of modulation of genes that are downstream of the cytokine IL-2; accordingly, CD8$^+$ T cells primed by hepatocytes are refractory to anti-PD-L1 treatment but can be rescued by interleukin-2. These findings reveal the dynamic behavior of naïve CD8$^+$ T cells undergoing intrahepatic priming and suggest potential strategies for the therapeutic restoration of dysfunctional CD8$^+$ T cells during chronic HBV infection.

S.E2 How to handle big data and can we do this?

S.E2.01
Human Systems Immunology - Cell by Cell

***J. L. Schultze**;*
LIMES-Institute, University of Bonn, Bonn, Germany.

During the last three decades, immunology was characterized by work mainly in murine model systems, gene knockout technologies and a focus on single pathways or single genes. However, to understand the immune system as a system, we actually have to develop multi-science approaches interacting and collaborating with experts from other fields including genomics, cell biology, bioinformatics, the computational sciences and even mathematics. Furthermore, with increasing knowledge about regulatory elements that are not evolutionary conserved between species, we need to switch to research in humans to better understand major human diseases. I will lay out and exemplify the path towards a truly human systems immunology strategy as the basis to better understand inflammatory conditions throughout the major human diseases. Single cell genomics technologies will play an important step forward to better describe and understand the role of certain immune cells in organ homeostasis, but even more so during the development and progression of major diseases. For example, by single cell RNA-sequencing, multi-color flow cytometry and functional testing we are currently mapping the human immune system in healthy and diseased lungs with highest resolution currently possible. Such approaches allow us now to define whether organ-resident immune cells or natural immigrants are major players during immunopathologies such as asthma or chronic obstructive pulmonary disease. Furthermore, the combination with therapeutic interventions allows us even to study immune system response under perturbation conditions. Collectively, the technological revolution in genomics towards single cell resolution will greatly impact on our possibilities to directly study disease in humans.

S.E2.02
How to make sense out of big data in immunology: The single cell genomics revolution as an example

***R. Zinkernagel**;*
University of Zurich, University Hospital, Zurich, Switzerland.

I shall try to illustrate how immunological research may be divided into 1) Surprising observations motivating experimental analysis or 2) Experiments begging for a question (P.Medawar). As long as we know little, new findings only rarely can be placed properly within a co-evolutionary context, because our methods of measurement are inadequate. Once we know almost everything any new detail (also from big data) can be interpreted much more adequately. Disease and death are excellent motivators for the analysis of aetiology and rate limiting steps by using adequate methods (including big data) as a starting point to improve our understanding of immunology, immunity and evolution. Big data analysis alone is a waste of money, but as a method will help to make observation-driven analysis quicker and medically helpful, if kept within an evolutionary context.

S.E2.03
How to make sense out of big data in immunology: The single cell genomics revolution as an example

***J. J. C. Neefjes**;*
Leiden University Medical Center, Leiden, Netherlands.

MHC class II molecules control many immune responses by presenting antigenic fragments acquired in the endosomal system to CD4+ T cells. Both tissue selective expression and the cell biology are complex and involve many different factors and systems. I will present how multi-dimensional screens can help placing proteins in pathways controlling MHC class II expression in an unbiased manner. This will be illustrated by a number of examples showing new pathways in control of MHC class II release in immature DC (effectively generating a mature DC phenotype), factors in control of MHC class II expression and the control of MHC class II expression in non-APC cells. In the latter case, a genetic and chemical screen was integrated that yielded both pathways and drugs in control of MHC class II expression with impact for associated diseases.

S.E4 Cell communication and signaling in the immune system

S.E4.01
Common and distinct Immune functions of exosomes and other extracellular vesicles

***C. Thery**, M. Tkach;*
INSERM U932, Institut Curie, Paris, France.

Cells secrete into their environment different types of extracellular vesicles (EVs) that have distinct properties depending on their intracellular site of origin. Exosomes are a subtype of EVs with a mean diameter lower than 150 nm that are formed inside multivesicular compartments of the endocytic pathway. Exosomes secreted by dendritic cells (DCs) have been shown to bear functional MHC class I and class II molecules able to activate cognate T lymphocytes and induce anti-tumor immune responses. These findings motivated the use of DC-derived exosomes in cancer clinical trials, although with limited clinical effects. Other EVs also bear functional immune molecules and may thus represent alternative immunotherapy tools. In our recently published work, we have isolated different subtypes of EVs simultaneously released by live human primary DCs to characterize their protein composition, and their abilities to activate T lymphocytes. We have observed that all EVs activate T cells as efficiently, but that the resulting functionality of T cells is different. Interestingly, exosomes were not the most efficient T-cell-activating EVs. Differences in the relative levels of surface co-stimulatory proteins in the different EV subtypes can explain differences of activities. We are now analysing the common and different abilities of EVs secreted by tumor cells to induce immune responses. Our results highlight the need to determine the respective roles of exosomes and other EVs, in cancer-immune system cross-talk but also in many other patho-physiological systems, to identify the best therapeutic or diagnostic EV-based tools.

S.E4.03
Metabolic programs controlling immune cell function

***T. Sparwasser**;*
Institute of Medical Microbiology and Hygiene, Johannes Gutenberg-University, Mainz, Germany.

Recent advances in the field of immunometabolism support the notion that essential processes in T cell biology, such as TCR-mediated activation and T helper lineage differentiation, are closely linked to changes in the cellular metabolic programs. Although the main task of the intermediate metabolism is to provide the cell with a constant supply of energy and molecular precursors for the production of biomolecules, the dynamic regulation of metabolic pathways also plays an active role in shaping T cell responses. Key metabolic processes such as glycolysis, fatty acid and mitochondrial metabolism are now recognized as crucial players in T cell activation and differentiation, and their modulation can differentially affect the development of T helper cell lineages. We only begin to understand the diverse metabolic processes that T cells engage during their life cycle from naïve towards effector and memory T cells. Many milestone discoveries in this active area of research are based on the use of chemical inhibitors that have been shown to possess off-target effects, emphasizing the importance of genetic models to study immunometabolism. Following activation, T cells switch to fatty acid synthesis, demonstrating that *de novo* lipid synthesis actively supports T cell proliferation and differentiation. We could show previously that pharmacological or genetic ACC1 inhibition impairs T helper cell induction, with the strongest impact on Th17 development. Here we discuss the molecular mechanisms that link metabolic changes with the control of gene expression.

JOINT SYMPOSIA

JS.01 Trends in Vaccinology

JS.01.02
Novel vaccines against old foes: Dengue, Zika, Ebola & Co

F. X. Heinz;
Medical University of Vienna, Center for Virology, Vienna, Austria.

Emerging viruses pose great challenges to global health and require enormous efforts for their control, as exemplified by the recent outbreaks of Ebola and Zika viruses and the continuous fight against dengue viruses, causing by far the highest number of arbovirus infections in tropical and subtropical regions worldwide. In addition to other means of outbreak control, vaccines cannot only contribute to manage acute emergencies but also provide long-term immunity to populations at risk. Probably the best example for a highly successful vaccine against an emerging virus is the live-attenuated yellow fever vaccine, which was developed ingeniously about 80 years ago in the absence of detailed knowledge of viral molecular biology, immunology and pathogenesis. Today, the armamentarium in the search for vaccines has increased impressively and led to the establishment of so-called vaccine platforms that can be readily exploited for developing vaccines against a variety of viruses, including Zika virus. These platforms include vector vaccines, recombinant immunogens produced in soluble or particulate forms, DNA and RNA vaccines, genetically engineered attenuated viruses, and chimeric viruses that can be used as live vaccines. Such chimeric replication-competent viruses are currently used and further evaluated as Ebola and dengue vaccines. Results have been encouraging, but especially in the case of dengue also provided evidence for potential negative side effects, related to an intrinsic problem of dengue pathogenesis that interferes with vaccine performance. New technologies like structure-based vaccine design may be starting points for developing more effective immunogens.

JS.01.03
A new generation of vaccines: for each target group its own adjuvant?

E. C. Lavelle;
Trinity College Dublin, Dublin, Ireland.

The effectiveness of vaccines and their capacity to promote and direct adaptive immunity depends on the induction of specific types of innate immune responses. As we increasingly adopt subunit vaccines which depend on adjuvants for their immunostimulatory potential, there is scope to refine vaccine formulations for specific conditions and defined target groups. Innovative adjuvant approaches can allow vaccines to be targeted at specific groups, for example the elderly or neonates. However, detailed knowledge of the nature of immune regulation in such groups and of the expression of specific innate immune sensors is required to facilitate rational vaccine design. Innate immune factors such as type 1 interferons and inflammasomes can play key roles in promoting adaptive immunity and identifying how adjuvants can regulate these and other such pathways which direct adaptive immunity can provide valuable targets for vaccine design. Adjuvants can also facilitate a move from injectable to oral vaccines which are attractive for the many enteric infections we are faced with. In conclusion, advances in our understanding of how innate immunity impacts on adaptive responses and in the design and formulation of adjuvants allows greater potential for a targeted approach to vaccination in future.

JS.02 Systems Immunology for stratifying patients with autoimmune diseases

JS.02.03
The Stratification of Lupus

M. Alarcòn Riquelme;
Center for Genomics and Oncological Research (GENYO), Granada, Spain.

Objectives: The highly heterogeneous clinical presentation of lupus is characterized by the unpredictable appearance of flares of disease activity and important organ damage. Attempts to stratify lupus patients have been limited to clinical information, leading to unsuccessful clinical trials and controversial research results. Our aim was to develop and validate a robust method to stratify patients with lupus according to longitudinal disease activity and whole-genome gene expression data in order to establish subgroups of patients who share disease progression mechanisms. **Methods**: We applied a clustering-based approach to stratify SLE patients based on the correlation between disease activity scores and longitudinal gene expression information. Clustering robustness was evaluated by bootstrapping and the clusters were characterized in terms of clinical and functional features. **Results**: Using two independent sets of patients, one pediatric and another adult, our results show a clear partition into three different disease clusters not influenced by treatment, race or other source of bias. Two of the clusters differentiate into a neutrophil correlated disease group and a lymphocyte correlated disease group, while the third that correlated to a lesser extent with neutrophils, was functionally more heterogeneous. The neutrophil-driven clusters were associated with increased development towards proliferative nephritis. **Conclusions**: We found three subgroups of patients that show different mechanisms of disease progression and are clinically differentiated. Our results have important implications for treatment options, the design of clinical trials, the etiology of the disease, and the prediction of severe glomerulonephritis.

JS.03 Antigen Presentation in Health and Disease

JS.03.02
Antigen processing and presentation in cancer immunosurveillance

R. Binder;
University of Pittsburgh, Pittsburgh, PA, United States.

Binder, Robert
University of Pittsburgh, Pittsburgh PA

The immune system recognizes aberrant cells and eliminates them prior to emergence of nascent tumors. This prevents progression of many malignancies. In the absence of such immunity in mice or humans, multiple and frequent tumors are generated. Current immunosurveillance model involves the priming of T cell and NK cell immunity. The gap in knowledge in this model is raised in two questions; (1) What is the molecular mechanism for cross-priming T cell responses in the context of the negligible amount of antigen available at the early stages of nascent tumor development? (2) What is the stimuli for co-stimulation of T cell priming and activation of NK cells. Both of these questions are unanswered. Our work has demonstrated that tumor-derived heat shock proteins (HSPs), introduced during vaccination, are super-efficient at cross-presentation of limited amounts of their chaperoned tumor (peptide) antigen. HSPs are also capable of initiating signals for co-stimulation. Both events require the HSP receptor, CD91, expressed on dendritic cells and together allow for priming potent tumor-specific T cells. The release of cytokines by DCs stimulated with HSPs enhances the T cell responses and activates NK cells. Our data shows that when tumor antigen levels are limiting, as in nascent emerging tumors, the HSP-CD91 pathway is essential for cross-priming of anti-tumor immune responses. The HSP-CD91 pathway represents a novel mechanism for initiating responses for cancer immunosurveillance.

JS.03.03
Generating peptide-MHC ligands for immune surveillance of foreign and self

N. Shastri[1,2], C. Park[1], J. Guan[1], T. Ding[1], F. Gonzalez[1];
[1]University of California, Berkley, CA, United States, [2]Johns Hopkins University School of Medicine, Baltimore, MD, United States.

The normal peptide repertoire presented by classical and non-classical MHC class I molecules is regulated by ERAAP, the endoplasmic reticulumaminopeptidase associated with antigen processing. Loss of ERAAP's peptide trimming function in cells causes dramatic changes in the peptide repertoire. The changes in the peptide repertoire enhance the immunogenicity of ERAAP-deficient cells and elicit potent immune responses in otherwise syngeneic wild-type mice. Because changes in ERAAP activity can cause abnormal immune responses, normal ERAAP function is monitored by an unusual subset of semi-invariant CD8+ T cells. These T cells recognize the QFL ligand that consists of a conserved peptide presented by the non-classical Qa-1^{b}MHC Ib molecule displayed only on surface of ERAAP-deficient cells. We show that these QFL-specific CD8+ T cells (QFL-T cells) bear unique and semi-invariant αβ TCRs. Genetic manipulation of the expression of the self-QFL ligand and functional characteristics of QFL-T cells shows that in addition to monitoring ERAAP function, QFL-T cells may also regulate metabolic activity.

JS.04 HLA in Transplantation and Autoimmunity

JS.04.03
HLA in Autoimmune Diseases

***L. M. Sollid**;*
Department of Immunology, Rikshospitalet, University of Oslo, Norway.

More than 50 years have passed since the first HLA association with disease was described, and in recent years genome wide association studies have taught us that HLA typically is the chief genetic determinant for autoimmune disorders. Yet, for most of the autoimmune diseases the underlying mechanism for their HLA association is elusive. There is strong linkage disequilibrium in the HLA gene complex making is hard to determine the primary HLA associations. Notwithstanding, for many diseases there is accumulating evidence that the primary associations are with classical HLA molecules. The principal role of these molecules to present peptides to T cells suggests an involvement of T cells in the pathogenesis. It is striking that autoimmune diseases with autoantibodies typically are primarily associated with HLA class II alleles, whereas seronegative diseases most often are associated with HLA class I alleles. HLA class II associations and presence of autoantibodies speak to involvement of B cells in addition to T cells. Celiac disease has a strong association to certain HLA-DQ allotypes and is hallmarked by very disease specific autoantibodies to the enzyme transglutaminase 2. I will present data on the molecular mechanisms for the HLA association of this disease highlighting the role of T cell and B cell interaction in the pathogenesis.

JS.05 Combinatorial approaches to develop targeted immunotherapeutics

JS.05.03
Novel insights into membrane targeting of B cell lymphoma: lessons learned from tetraspanins

***A. van Spriel**;*
Radboud university medical center, Nijmegen, Netherlands.

The plasma membrane of immune cells contains thousands of different proteins including receptors, enzymes and signalling molecules. A tight spatiotemporal organisation of these membrane proteins is essential for immune cell function including pathogen recognition, antigen presentation and migration. The superfamily of tetraspanin proteins plays a central role in organising membrane proteins and signalling molecules into the 'tetraspanin web'.
We discovered that tetraspanins CD37 and CD53 control B cell function. *Cd37*-deficient mice have reduced numbers of IgG+ antibody-secreting cells in lymphoid organs, which is a B-cell intrinsic defect as demonstrated in chimeric mice. In contrast, IgA+ B cell numbers are increased, and deficiency of CD37 induces the spontaneous development of B-cell lymphoma in vivo. We identified that CD37 interacts with suppressor of cytokine signalling 3 (SOCS3), and when absent drives tumour development through constitutive activation of the IL-6 signalling pathway. Moreover, loss of CD37 on neoplastic cells in patients with diffuse large B-cell lymphoma is directly correlated with worse progression-free and overall survival.
Together, this study identifies CD37 as novel tumour suppressor that protects against B cell lymphomagenesis, and provides a strong rationale for blocking the IL-6 pathway in patients with CD37-negative B cell malignancies as therapeutic intervention.
De Winde, Veenbergen et al. *J. Clin. Invest*. 2016;126(2):653.
De Winde et al. *Trends in Cancer* 2017;3(6):442.
This work is supported by the Dutch Cancer Society (KUN2014-6845), Netherlands Organization for Scientific Research (NWO-VIDI Grant 864.11.006), and the ERC (Consolidator Grant, 724281).

JS.07 Subversion of phagocytosis in innate immunity: from efferocytosis to pathogen interaction

JS.07.01
Neutrophils as targets for host-directed therapy against tuberculosis

***T. Dallenga**[1,2], U. Repnik[3], R. Reimer[4], G. Griffiths[5], U. E. Schaible[1,2];*
[1]Research Center Borstel, Borstel, Germany, [2]TTU-TB, German Center for Infection Research, Munich, Germany, [3]Max Planck Institute of Molecular Cell Biology & Genetics, Dresden, Germany, [4]Heinrich Pette Institute, Hamburg, Germany, [5]University of Oslo, Oslo, Norway.

With multi drug-resistant *M. tuberculosis* variants on the rise, novel approaches to tackle the global tuberculosis epidemic are needed. Neutrophils represent the main infected cell population in lungs of patients with active tuberculosis. Here we show that *M. tuberculosis* induces necrosis of human neutrophils in an ESX-1-dependent manner. Necrosis was a prerequisite for mycobacterial growth in human macrophages after subsequent removal of infected, necrotic neutrophils. After identification of reactive oxygen species (ROS) as drivers of necrosis, we were able to prevent necrosis by pharmacological inhibition of myeloperoxidase. Thereby, we restored the capability of efferocytic macrophages to control mycobacterial growth, highlighting ROS and ROS-producing enzymes as putative targets for host-directed therapy. Taken together, host cell necrosis represents the starting point for a vicious circle leading to subsequent uptake of infected necrotic cells by other phagocytes, mycobacterial growth therein and, again, induction of host cell necrosis, a scenario that is very likely to take place in patients. Interruption of this vicious circle by inhibition of necrosis and subsequent restoration of the anti-mycobacterial functions represent an intriguing approach for host-directed therapy.

JS.07.02
Differential signaling through TLR7 or TLR8 determines the phenotype of human monocytes during RNA viral infection

*M. de Marcken, K. Dhaliwal, A. Gautron, **M. Dominguez-Villar**;*
Yale University, New Haven, CT, United States.

Despite being one of the major cellular targets of many RNA
virus infections in peripheral blood, the early events that occur after interactions between human monocytes and RNA viruses, and the signaling pathways responsible for the activation of these cells during infection are not well understood. Toll-like receptors (TLR) are a major family of pattern recognition sensors that trigger specific activation pathways in cells of both the innate and adaptive arms of the immune system. There are at least 10 TLRs in humans, from which TLR7 and TLR8 recognize single-stranded RNA. Despite both recognizing the same generic ligand, we and others have demonstrated different phenotypic outcomes on cells stimulated through either TLR7 or TLR8. We have recently observed fundamental differences in the phenotype and function of monocytes stimulated via either TLR7 or TLR8, in the context of RNA virus infections, and specifically, in terms of type I IFN responses and effector cytokines they produce, as well as general differences in cell surface markers. We have defined the molecular mediators that are responsible for these differences in phenotype by performing *ex vivo* experiments with human monocytes isolated from blood and we have shown the relevance of these data in common RNA virus infections, demonstrating that TLR7 and/or TLR8 stimulation by RNA virus infections of human monocytes account for much of the phenotype the cells acquire upon virus interaction.

JS.07.03
Efferocytosis induces a novel SLC program to promote glucose uptake and lactate release

***J. S. A. Perry**[1], S. Morioka[1], M. H. Raymond[1], C. B. Medina[1], L. Zhou[2], V. Serbulea[1], S. Onengut-Gumuscu[1], N. Leitinger[1], J. C. Rathmell[3], L. Makowski[3], K. S. Ravichandran[1];*
[1]University of Virginia, Charlottesville, VA, United States, [2]University of North Carolina, Chapel Hill, NC, United States, [3]Vanderbilt University, Nashville, TN, United States.

On a daily basis, we turnover billions of apoptotic cells that are removed by professional and non-professional phagocytes. While characterizing the transcriptional program of phagocytes, we discovered a novel solute carrier family (SLC) gene signature (33 SLC members) that is specifically modified during engulfment of apoptotic cells (efferocytosis) but not during antibody-mediated phagocytosis. When we assessed the functional relevance of these SLCs, we noted robust induction of an aerobic glycolysis program in engulfing phagocytes, initiated by SLC2A1-mediated glucose uptake, and suppression of oxidative phosphorylation program. Interestingly, the different steps of phagocytosis, i.e. smell ('find-me' signals / sensing factors released by apoptotic cells), taste (phagocyte- apoptotic cell contact), and ingestion (corpse internalization), activated different molecules to promote this glycolytic process. Further, lactate, a natural byproduct of aerobic glycolysis, was released from engulfing phagocytes via SLC16A1, an SLC member activated after corpse uptake. While glycolysis within phagocytes was needed for the continued uptake of corpses, the lactate released via SLC16A1 contributed to the establishment of an anti-inflammatory environment. Collectively, these data reveal a novel SLC program activated during efferocytosis, identify a previously unknown reliance on aerobic glycolysis during apoptotic cell uptake, and show that glycolytic byproducts of efferocytosis can also influence other cells in the microenvironment.

JS.08 Cytometry building bridges

JS.08.01
Cytometry of aging of the immune system

***A. Cossarizza**;*
University of Modena and Reggio Emilia, Modena, Italy.

In most developed and rich countries, human life span has dramatically increased because of great advances in preventing, delaying, or curing several pathologies. In all mammals, the aging process is characterized by profound changes in immunological responses that can be identified either analyzing the phenotype of lymphocytes (typically, collected from peripheral blood in the case of donors) or their functionality. For these purposes, several techniques have been developed in the last decades, but with no doubts flow cytometry has assumed the main role, due to the possibility to analyze several parameter (at present, up to 27 by using fluorescent dyes, more than 40 by mass cytometry) at the single cell level. Thus, a very fine characterization of age-related changes is occurring, and, for example, researchers are clarifying the importance of exhaustion markers among different subsets of T cells as predictors of morbidity and mortality, building a new immune risk profile. Not only cytometry is now allowing to identify such a huge amount of parameters, but also to identify rare cells, i.e. those present in a percentage that is much less than 1 in one thousand, like those specific for a given antigen. So, for example, the identification of responding cells is crucial for a better understanding of the effect of vaccines in the elderly. The talk will present some recent advancement in the field of immunology of aging that have been obtaining by studies at the single cell level.

JS.08.03
Standardizing cytometry of primary immunodeficiencies

***T. Kalina**;*
Charles University, Prague, Czech Republic.

EuroFlow consortium has developed and validated an set of 8-color flow cytometry panels for the initial evaluation of patients with the clinical suspicion of a Primary Immunodeficiency (PID). We have analyzed 96 PID patients and additional 21 severe PID diagnosed until the age of 2 years.
I will aim to illustrate following objectives:
•How and why to do standardized measurements in an international collaboration
•Investigation of lymphocyte subsets (incl. their maturation status) in one tube can be used to asses abnormalities in primary immunodeficiency
•Approach to comprehensive visualisation of results
•Search for hallmarks
•Present an example of diagnostic utility of the two tube test for sever PID (SCID, Omenn syndrome, CID and other PIDs with molecular lesion at the age 0-2 years)
In summary, out of the 96 PID patients we failed to uncover lymphocytes' subsets abnormality only in 2 patients with Chronic Granulomatous Disease and 4 patients with complement deficiencies (in line with literature), but we were able to find at least one abnormality in all remaining patients with PID. Furthermore the T-cell directed tube revealed accurately all SCID and Omenn syndrome patients even in cases with maternal engraftment. This is the first approach to a standardized PID diagnostic test that should reveal all alterations in patient lymphocytes' compartment without a correct a priori assumption about the PID nature (a universal test).
Acknowledgement: Supported by EuroFlow and a grant 15-28541A and LO1604.

JS.09 The gut microbiota and the IgA antibody production in health and disease

JS.09.03
The regulatory microenvironment in Peyer's patches leading to synchronized gut IgA responses

***N. Lycke**[1,2], M. Bemark[2], R. Komban[2], A. Strömberg[2], Z. Shulman[3];*
[1]Gothenburg, Sweden, [2]University of Gothenburg, Gothenburg, Sweden, [3]The Weizmann Institute of Science, Rehovat, Israel.

The majority of activated B cells differentiate into IgA plasma cells at mucosal sites, with the gut being the largest producer of immunoglobulin in the body. Secretory IgA antibodies have numerous critical functions of which protection against infections and the role for establishing a healthy microbiota appear most important. Expanding our knowledge of the regulation of IgA B cell responses and how effective mucosal vaccines can be designed are of critical importance. I will discuss recent developments in this field that shed light on the uniqueness and complexity of the gut mucosal IgA inductive site, the Peyer's patches. In particular, I will describe a novel B cell dependent pathway for bringing luminal antigens from the M cell to the germinal centers in the Peyer's patches. In addition, single cell RNAseq data on the composition of antigen-specific B cells in the Peyer's patches following oral immunizations will be presented.

JS.10 Innate host pathogen interactions

JS.10.01
Protective role of Mincle in Gram-positive bacterial infection

***S. Yamasaki**;*
Osaka University, Osaka, Japan.

C-type lectin receptors (CLRs) comprise a large family of proteins that share a common structural motif and are involved in various immune responses. Among them, ITAM-coupled CLRs are recently identified as pattern recognition receptors (PRRs) for pathogens. Mincle (Macrophage-inducible C-type lectin) is an FcRγ-coupled activating receptor that recognizes mycobacterial glycolipid, trehalose dimycolate, to promote protective immunity against mycobacteria. Recently, we found that Mincle also recognizes Gram-positive bacterial pathogen, Group A *Streptococcus* (GAS). GAS causes fatal invasive infections and thus called "flesh-eating bacteria"; however, the mechanism by which our immunity react this pathogen is not well understood. Within GAS components, we purified and identified unique glycolipids as Mincle ligands. Upon invasive GAS infection, Mincle-deficient mice exhibited impaired cytokine production of pro-inflammatory cytokines, severe bacteremia and rapid lethality. These results indicate that Mincle plays a central role in protective immunity against acute GAS infection.

JS.10.02
LILR family receptors in host pathogen interactions

***H. Arase**[1,2];*
[1]Research Institute for Microbial Diseases, Osaka University, Suita, Japan, [2]Immunology Frontier Research Center, Osaka University, Suita, Japan.

Immune cells express various kinds of paired receptors that consist of inhibitory and activating receptors. Although their extracellular domains are highly homologous between inhibitory and activating receptors, inhibitory receptors possessing ITIM at cytoplasmic domain downregulate activation of immune cells, whereas activating receptors deliver activating signals via ITAM positive adaptor molecules like DAP12. LILR family receptors are one of representative paired receptors mainly expressed on innate immune cells.
From the analysis of LILR family receptors, we found that inhibitory LILRB1 is used by *Plasmodium falciparum* for immune evasion. Furthermore, immune evasion of *Plasmodium falciparum* via LILRB1 was associated with severe malaria (*Nature* 2017). On the other hand, we have found that LILRA2, one of activating LILR family receptors, specifically recognizes immunoglobulin cleaved by bacterial protease whereas LILRA2 does not recognize normal immunoglobulin. This suggested that activating LILRA2 plays an important role in host defense by sensing immunoglobulin abnormalities (*Nature Microbiology* 2016). These studies suggest that LILR family receptors play an important role in host-pathogen interaction.

JS.10.03
ILC2 and type 2 immune diseases

***K. Moro**;*
RIKEN IMS, Yokohama, Japan.

Recent studies have revealed new types of lymphocytes functioning in innate immune responses that are collectively called innate lymphoid cells (ILCs). Unlike T and B lymphocytes, ILCs lack Rag-dependent antigen-specific receptors and are activated by cytokines produced by other innate immune cells or epithelial cells. ILCs have been divided into 3 groups based on their cytokine production profiles; group 1 ILC including NK cells and ILC1 produce IFNγ, group 2 ILC (ILC2) including natural helper cells, nuocytes and innate helper type 2 cells produce type 2 cytokines such as IL-5, IL-6 and IL-13, and group 3 ILC including lymphoid tissue inducer (LTi) cells and ILC3s produce IL-17 and IL-22. ILCs play important roles in protection against various invading microbes including multicellular parasites, and in the maintenance of homeostasis and repair of epithelial layers. ILC2 produce a large amount of IL-5 and IL-13 in response to IL-25 or IL-33, and induce eosinophilia and goblet cell, both of which act to protect against helminth infection and exacerbation of allergy. Since we discovered ILC2 in 2010, many other research groups have joined this research field and identified new immune responses that are regulated by ILC2. In particular, the importance of ILC2 in allergic diseases has received a fair amount of attention and new evidence indicates that allergic disorders occur not only from allergen-specific pathways but are also induced by allergen non-specific pathways due to ILC2 activation.

EDUCATIONAL SESSIONS

EDU.01 Systems Biology for Immunology: Help with the Complexity

EDU.01.03
Systems Immunology: Making sense of the Yins and Yangs

H. V. Westerhoff*;*
AIMMS and SILS and MCISB, Amsterdam and Manchester, Netherlands.

Thousands of molecules are discussed at this conference. The good news is that most can now be measured quantitatively: Immunology must thereby be the most challenging science for which most of the building blocks of complexity can be measured. How can they be understood however? Will the Biology that draws diagrams and arrows, suffice? The interactions between immunological factors and phenotype or disease appear to be determined by multiple networks that work positively and multiple that work negatively. For what we want, i.e. to understand and then cure, the usual schemes with plusses and minuses won't do. In an analogy with Deep Sequencing, this conference brings 'Deep Immunology' to the fore, i.e. a strategy that collects 'all' data and then puts these into a 'machine' that helps understand what they imply. Such a 'machine' is a new type of mathematical model, i.e. a 'watchmaker model', which is able to assimilate all mechanisms into a coherent and predictive whole; a whole that may not be greater than the sum of its parts, but actually smaller (simpler), and certainly different from that sum. This presentation will present one such model, which deals with multiple concatenated regulatory networks of various signs and strengths that affect the innate immune response. It will show how the model gives a handle on understanding the dichotomy between acute and chronic inflammation and what this has to do with supplementation of stem cells in immune therapies. And it will show how immunologists can now use this Deep Immunology.

EDU.02 The Utility of Theories in Immunology

EDU.02.02
The "two-niche" theory of T cell Memory

F. Di Rosa*;*
Institute of molecular biology and pathology, Italian National Research Council (CNR), Rome, Italy.

The concept is emerging that the bone marrow (BM) sustains life-long persistence of memory T cells, as it does for long-lived plasma cells. The majority of BM memory CD8 T cells are recirculating cells in constant exchange with blood, although some may permanently reside in the BM, thus resembling tissue-resident memory CD8 T cells of extra-lymphoid organs (e.g. gut, skin, etc.). Indeed, it has been suggested that the BM provides "niches" for memory CD8 T cell self-renewal, thus controlling the maintenance of memory CD8 T cell number in the body. However, many gaps remain unfilled, and in particular how memory CD8 T cells are steadily maintained as seemingly quiescent cells, and yet are poised to promptly generate a huge progeny of effector CD8 T cells upon secondary response. In an active debate on the regulation of memory T cell quiescence, I have built upon my own and others' data to propose a novel hypothesis: namely, that the BM offers two types of niches for memory T cells: one driving self-renewal, the other supporting quiescence, thus echoing the duality of niches for hematopoietic stem cells. While self-renewal would stably preserve memory T cell numbers, maintenance in a quiescent state would preserve a capacity to promptly mount secondary responses. Hence, testing this hypothesis is key to considering how CD8 T cell responses to discrete challenges may be beneficially manipulated.

EDU.02.03
The Discontinuity Theory of Immunity

E. Vivier[1]*, T. Pradeu*[2]*;*
[1]*Aix Marseille Univ, CNRS, INSERM, APHM, CIML & Innate Pharma, Marseille, France,* [2]*Unité d'immunologie (ImmunoConcEpT, UMR5164), Université de Bordeaux, France.*

Similar to many other biological systems, the immune system can be seen as a change-detection system. According to the **discontinuity theory of immunity**, the immune system responds to sudden changes in antigenic stimulation and is rendered tolerant by slow or continuous stimulation. The immune system can adapt to these slow or long-lasting modifications in the host, which it then treats as a new reference point. This basic principle, which is supported by recent data on immune checkpoints in viral infections, cancers, and allergies, can be seen as a unifying framework for diverse immune responses. The mechanisms underlying the recognition of patterns, the absence of a pattern, tissue damage, and functional modifications have been considered separately, but the **discontinuity theory of immunity** explains these mechanisms as instances of the more general rule that immune systems have been selected by evolution to respond to sudden modifications within the host.

EDU.02.04
The Equilibrium Model of Immunity

G. Eberl*;*
Institut Pasteur, Paris, France.

The classical model of immunity states that the immune system reacts to pathogens and injury and restores homeostasis. Indeed, a century of research has uncovered the means and mechanisms by which the immune system recognizes danger and regulates its own activity. However, this classical model does not fully explain complex phenomena, such as tolerance, allergy, the increased prevalence of inflammatory pathologies in industrialized nations and immunity to multiple infections. I propose a model of immunity that is based on equilibrium, in which the healthy immune system is always active and is in a state of dynamic equilibrium between antagonistic types of response. This equilibrium is regulated both by the internal milieu and by the microbial environment. As a result, alteration of the internal milieu or microbial environment leads to immune disequilibrium, which determines tolerance, protective immunity and inflammatory pathology.

EDU.03 Immunology of extracellular vesicles

EDU.03.01
Extracellular vesicles in acute and chronic Inflammation

E. I. Buzàs*;*
Semmelweis University, Budapest, Hungary.

Extracellular vesicles are phospholipid membrane enclosed structures released by cells in an evolutionarily conserved manner. These subcellular structures are secreted even under steady state conditions as part of the homeostatic cell-cell communication. Importantly, upon cell activation, induction of cellular stress or different types of cell death, both the number and the molecular composition of extracellular vesicles are altered. In innate and adaptive immune reactions, extracellular vesicles convey intercellular messages by delivering their molecular cargo (proteins, lipids, nucleic RNA and DNA and metabolites). Extracellular vesicles are important carriers of danger signals. There are numerous examples showing that PAMPs and DAMPs are associated with extracellular vesicles and are recognized by the innate immune system. Furthermore, by carrying a wide variety of cytokines and surface-associated tissue degrading enzymes, extracellular vesicles are important players in both acute and chronic inflammation. They have the ability to stimulate antigen-specific T cells, and they participate in cross-dressing and cross-presentation of APCs. The role of extracellular nuclear molecules in the pathogenesis of autoimmune diseases is increasingly recognized. Rapidly growing number of data supports that extracellular vesicles are complex packages of autoantigens which often represent the focus of autoimmune reactions. Finally, they are not only promising biomarkers of diseases but also have significant therapeutic potential in diseases with immune pathomechanism.
Grants: NVKP_16-1-2016-0017, OTKA11958 & 120237, VEKOP-2.3.2-16-2016-00002 and VEKOP-2.3.315201600016, the Institutional Higher Education Excellence Program of the Ministry of Human Resources in the theme "Therapeutic development" and H2020-MSCA-ITN TRAIN-EV.

EDU.03.02
Isolation and detection of extracellular vesicles

***R. Nieuwland**;*
Amsterdam UMC, Amsterdam, Netherlands.

Human body fluids contain cell-derived membrane-enclosed vesicles. The cellular origin, concentration, composition and function of vesicles in body fluids differ in health and disease. Therefore it is not surprising that the scientific and clinical interest in EVs is growing exponentially because vesicles potentially behold the promise of an entirely new set of biomarkers.
Body fluids contain multiple types of vesicles, which are involved in intercellular communication, cellular waste management, and host defence (inflammation, coagulation). Because the types of vesicles are difficult to distinguish from each biochemically (composition) or biophysically (size, density), the umbrella term “extracellular vesicles” was introduced by the International Society of Extracellular Vesicles (ISEV).
Most EVs in body fluids are extremely small and spherical, and the bulk of EVs have a diameter of less than 200-300 nm. Blood, which is the most commonly studied body fluid for biomarker research, contains not only EVs but also high concentrations of soluble proteins and lipoprotein particles / chylomicrons which overlap in size and density with EVs. Consequently, the isolation, detection and biochemical characterization of EVs is not trivial, and only recently several major methodological pitfalls and hurdles have been overcome.
In this presentation, an overview will be given about the progress that has been made regarding isolation of EVs, and the detection of single EVs by flow cytometry.

EDU.03.03
Extracellular vesicles in immunity

***S. Gabrielsson**;*
Karolinska Institutet, Dept of Medicine, Stockholm, Sweden.

Exosomes from antigen presenting cells are interesting as potential cancer immunotherapy vehicles due to their capacity to stimulate tumor-specific activity in mice. However, clinical trials using peptide-loaded autologous exosomes showed only moderate T cell responses, suggesting a need for optimization of exosome-based therapy. We are using dendritic cell derived exosomes to induce antigen-specific immune responses with the aim to cure cancer. We have seen that exosomes need to carry whole protein, and not only peptide/MHC complexes, to induce a strong T cell response in vivo. We showed that the reason for this was that B-cells needed to be activated to induce a strong T cell response to exosomes. This has led us to test allogeneic exosomes in a B16 melanoma model, and our results demonstrate that allogeneic exosomes are as efficient in inducing immune responses as syngeneic exosomes. This greatly increases the feasibility of exosome-based immunotherapy. Currently we are working on different ways to further boost immunogenicity of exosomes and data from these studies will be presented.

EFIS PRESIDENT'S SYMPOSIUM

EP.01 T Cell immunity in the front line

EP.01.03

Differentiation of tissue-resident memory T-cells in primary and secondary immune responses

K. van Gisbergen*;*
Sanquin Research, Amsterdam, Netherlands.

Tissue-resident memory T-cells (Trm) form populations of memory T-cells in barrier tissues including the epithelial compartments of lungs, skin, and gut. Trm are emerging as one of the most potent immune weapons against reinfection, prompting great interest in the development of therapeutic strategies for their elicitation. We have previously identified Hobit as a central regulator of Trm differentiation in mice. We found that the transcription factor was universally expressed in Trm populations of the skin, gut and liver, but not in circulating memory populations. Hobit together with related Blimp-1 was essential for the formation of Trm through suppression of the expression of tissue exit receptors such as S1PR1 and CCR7. Therefore, our findings indicate that Hobit and Blimp-1 mediate a universal program of tissue-residency. The restricted expression pattern of Hobit within resident lymphocytes is a characteristic that we deemed highly valuable for the development of novel tools to study Trm. Therefore, we generated a transgenic mouse model that contained a "knock-in" within the Hobit locus of an "all-in-one" construct of the tdTomato reporter, CRE recombinase and the diphtheria toxin receptor (DTR). We confirmed that expression of tdTomato and functional activity of the CRE recombinase and the DTR was nearly uniformly present and largely restricted to Trm in the transgenic mice. These findings suggest that we have established a novel model system to address Trm development *in vivo*. We aim to resolve the development and the potential of Trm through the further characterization of T-cell differentiation in Hobit reporter mice.

LATE BREAKING HOT TOPICS

HT.04 Late Breaking Hot Topic 4

HT.04.01

Smoking induces recruitment of monocytes into the alveolar space and contributes to accelerated growth of Mycobacterium tuberculosis

B. Corleis[1], *J. L. Cho*[2], *A. Linder*[1], *A. Yan*[1], *A. Dickey*[1], *A. E. Schiff*[1], *B. D. Medoff*[2], *D. S. Kwon*[1];
[1]*Ragon Institute of MGH, MIT, and Harvard, Cambridge, United States,* [2]*Division of Pulmonary and Critical Care Medicine, Massachusetts General Hospital, Boston, United States.*

20-30% of active tuberculosis (TB) cases worldwide are associated with tobacco smoking. Extensive monocyte recruitment in small animal models of human TB is associated with a higher total lung bacterial burden, suggesting that monocyte-derived macrophages may be more permissive to *Mycobacterium tuberculosis* (*M tb*) growth relative to resident macrophages in the lung. We recruited healthy current- and never-smokers for collection of bronchoalveolar lavage (BAL). Monocytes, BAL macrophages or monocyte-derived macrophages (MDMs) were analyzed using flow cytometry, and infected with *M tb in vitro* and bacterial growth monitored over time by CFU. We examined numbers of macrophages, T cells and granulocytes in BAL and found a significant increase in a population of small macrophages in BAL from smokers compared to non-smokers. Half of the small macrophages expressed surface CD93, a marker which distinguished circulating monocytes from large alveolar macrophages and suggests that these small macrophages are derived from newly recruited monocytes. BAL fluid from smokers recruited blood monocytes *in vitro* and significantly higher concentrations of the chemokine CCL11 in BAL fluid correlated with the number of CD93+ small macrophages in BAL. Virulent *M tb* induced a hyper-inflammatory response in human BAL monocytes with significantly higher intracellular growth compared to MDMs or large alveolar macrophages *in vitro.* In conclusion, our data indicate that smoking leads to higher numbers of total BAL macrophages due to CCL11 mediated recruitment of circulating CD93+ monocytes into the alveolar space. Importantly, monocytes were highly susceptible to M tb intracellular growth, suggesting that extensive monocyte infiltration plays a significant role in smoking associated risk for active tuberculosis.

HT.05 Late Breaking Hot Topic 5

HT.05.01

Quantitative shotgun proteo-/transcriptomics shows how human regulatory T cells protect their identity

D. Amsen[1,2];
[1]*Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands,* [2]*University of Amsterdam, Amsterdam, Netherlands.*

Inflammation is both a requirement and a challenge for regulatory T cell (Treg) function. Inflammatory cues direct Tregs to inflamed sites where they limit tissue destruction and promote repair. Under pathological conditions, inflammation can however coerce Tregs into assuming functions normally performed by conventional T cells (Tconvs), such as production of pro-inflammatory cytokines. Here, we have studied how Tregs generally protect their identity from such destabilization. For this, we first established a molecular definition of Treg identity by performing whole cell shotgun proteomics and transcriptomics on multiple human populations of Tregs and Tconvs. We found that proteome and transcriptome compositions markedly differ from one another, but are complementary, underscoring the importance of analysis at both levels. Core and subset-specific Treg signatures reveal that these cells have specific adaptations in cytokine-, TCR- and costimulatory receptor signaling pathways. We show that strategic deficiencies in individual pathways allow inflammatory cytokines to mobilize select functions in Tregs (such as expression of transcription factors and homing receptors) without compromising Treg identity. Genetic alterations in individual signature molecules however suffice to allow conversion of Tregs into Tconvs by inflammatory cytokines. Our results therefore identify molecular nodes that determine the unique response characteristics of Tregs to inflammation.

HT.06 Late Breaking Hot Topic 6

HT.06.01

A cautionary note for using pharmacological inhibitors challenges the role of long-chain fatty acid oxidation on immune cells

L. Berod[1], *B. Raud*[1], *D. G. Roy*[2], *A. S. Divakaruni*[3], *T. N. Tarasenko*[4], *R. Franke*[5], *M. Brönstrup*[5], *A. N. Murphy*[6], *P. J. McGuire*[4], *R. G. Jones*[2], *T. Sparwasser*[1];
[1]*Twincore, Hannover, Germany,* [2]*Goodman Cancer Research Centre, McGill University, Montreal, Canada,* [3]*David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, United States,* [4]*National Human Genome Research Institute, NIH, Bethesda, United States,* [5]*Helmholtz Centre for Infection Research, Braunschweig, Germany,* [6]*University of California, San Diego, La Jolla, United States.*

Long-chain fatty acid oxidation (LC-FA0) has been suggested to play an important role supporting CD4+Foxp3+ regulatory T cells as well as CD8+ memory T cell differentiation and survival. Similarly, LC-FAO has been associated with IL-4-driven macrophage polarization towards the alternative M2 phenotype. However, previous research leading to these conclusions is based on the pharmacological inhibition of carnitine palmitoyltransferase-1, the rate-limiting enzyme for LC-FAO, with high concentrations of the drug etomoxir. Using genetic mouse models to target Cpt1a on specific immune cell populations, we dissected the role of LC-FAO in primary, memory, and regulatory T cell responses. Challenging previous concepts, we here show that LC-FAO and Cpt1a are largely dispensable for effector, memory, or regulatory T cell formation, and that the effects of high dose etomoxir on immune cell differentiation and function are independent of Cpt1a expression. Together our data argue that metabolic pathways other than LC-FAO fuel CD8+ memory or Treg differentiation and suggest off-target effects of etomoxir on mitochondrial respiration.

BRIGHT SPARKS WORKSHOPS

BS.A.01 Bright Sparks A

BS.A.01.01
Developmental origin of osteoclasts and their functional maintenance by nuclear chimerism

E. Mass[1,2], *C. E. Jacome-Galarza*[2], *G. I. Percin*[3,4], *T. Lazarov*[2], *J. Eitler*[3,4], *M. Rauner*[4], *L. Crozet*[2], *M. Bohm*[2], *C. Waskow*[3,4], *F. Geissmann*[2];
[1]*Life & Medical Sciences Institute (LIMES), Bonn, Germany,* [2]*MSKCC, New York, United States,* [3]*Institute for Immunology, Dresden, Germany,* [4]*Leibniz-Institute on Aging-Fritz-Lipmann-Institute, Jena, Germany.*

Osteoclasts are multinucleated macrophages that continuously remodel the bone marrow hematopoietic niche. Excess osteoclast activity contributes to bone loss and osteoporosis, while decreased activity leads to osteopetrosis and bone marrow failure. Osteopetrosis can be partially treated by bone marrow transplantation in human and mice, which is in accordance with in vitro studies suggesting that osteoclasts develop by fusion of hematopoietic stem cell (HSC)-derived monocytic precursors. However, the developmental origin and lifespan of osteoclasts, and the mechanisms that ensure their maintenance and function in vivo remain largely unexplored. Here we report that osteoclasts are long-lived cells that originate from erythro-myeloid progenitors (EMP) in the yolk sac. Using genetic fate-mapping and knockout models we show that EMP-derived osteoclasts colonize ossification centers during embryogenesis and are required for normal bone development and teeth eruption. However, after birth osteoclasts depend on HSC-derived cells. Parabiosis and transfer experiments indicate that an iterative fusion of osteoclasts with circulating monocytes sustains their function is thus indispensable for the maintenance of the bone marrow niche during aging. Altogether, our results identify a dual origin of osteoclasts in vivo, and nuclear chimerism as a mechanism that enables long-term maintenance and function of these multinucleated cells, thereby suggesting new strategies to modulate osteoclast activity.

BS.A.01.02
Uncovering the regulatory T cell transcriptional signature in the human thymus

M. Ângelo-Dias*, A. Godinho-Santos, Y. Tokunaga, A. Serra-Caetano, H. Nunes-Cabaço, A. E. Sousa, A. A. Raposo;*
Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal.

Thymic committed regulatory CD4 T cells (Treg) are essential to maintain self-tolerance and immune homeostasis, yet there are no genome-wide data defining their expression profile in the human thymus. Although FoxP3 is known as a master regulator of Treg differentiation and function, many other factors remain to be uncovered.
To this purpose, we sorted mature CD4 single-positive thymic Tregs (tTreg) and their conventional counterparts (tTconv) based on the expression of CD27, CD25, and CD127, from three human thymuses collected during routine corrective pediatric cardiac surgery, and generated their respective expression profiles by RNA-seq.
Our comparative transcriptomic analysis identifies 1047 genes significantly differentially expressed between tTreg and tTconv subsets. We confirm the prominent expression of Treg associated genes (*FoxP3, IL2RA, CTLA4, LRRC32*, *IKZF2, IKZF4)* in tTreg relative to tTconv. Of note, 45 amongst the 648 tTreg highly-expressed genes encode for known transcription factors, including those involved in T cell activation and differentiation (*RORA, AHR, TBX21, STAT4*), and NF-kB pathway (*NFKB2*, *RELB*, and *REL*). To identify novel factors and pathways, we selected 196 transcripts uniquely expressed in tTreg and not expressed in tTconv. Remarkably, we uncovered groups of genes with a strong statistical association with the regulation of cell migration *(FN1, CCL22, LMNA, LAMA2, ICAM1, CXCR3)*, cytokine pathways (*CCR8, IRF5, TNFRSF8, IL12RB2, IL1RL1, EBI3*), and ion homeostasis (*RYR1, ACTN2, HMOX1, CHRNA6, TMPRSS6, CAV1, KCNS3, CASQ1*).
Our data open several new lines of research to further clarify pathways of Treg lineage commitment in the human thymus, and determinants of the human tTreg signature.

BS.A.01.03
Human T-bet$^+$ B cells: induction and effector functions from a multiple sclerosis perspective

J. van Langelaar[1], *L. Rijvers*[1], *M. Janssen*[1,2], *I. de Groot*[1], *L. Kalden*[1], *A. F. Wierenga-Wolf*[1], *S. Koetzier*[1], *T. A. Siepman*[2], *H. E. de Vries*[3], *P. Unger*[4], *M. S. van Ham*[4], *R. Q. Hintzen*[1,2], *M. M. Luijn*[1];
[1]*Dept. Immunology, MS Center ErasMS, Erasmus MC, Rotterdam, Netherlands,* [2]*Dept. Neurology, MS Center ErasMS, Erasmus MC, Rotterdam, Netherlands,* [3]*Dept. Molecular Cell Biology and Immunology, VU University Medical Center, Amsterdam, Netherlands,* [4]*Dept. Immunopathology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands.*

In multiple sclerosis (MS), peripheral B-cell tolerance checkpoints are defective and meningeal B-cell follicle-like structures are present in the central nervous system (CNS). Both these critical events link to the function of IFN-γ-induced autoreactive T-bet$^+$ B cells in mice. However, the exact B-cell subpopulations and mechanisms contributing to disease activity in MS patients are underexplored. Here, we found increased STAT1 phosphorylation in IFN-γ-triggered human B cells carrying a recently identified coding MS risk variant, *IFNGR2*. In 3T3-CD40L/IL-21 based cultures, B cells from MS patients revealed higher T-bet and enhanced ASC differentiation in the presence of IFN-γ compared to matched controls. When CpG-ODN was added, T-bet and CXCR3 were further upregulated and caused enhanced switching to IgG1. *Ex vivo* FACS analysis of B cells showed that CXCR3$^+$IgG$^+$ and not naive or non-class-switched cells were reduced in untreated MS versus control blood and were enriched in paired cell suspensions from MS brain tissue, meninges and cerebrospinal fluid. CXCR3 and not CXCR5, was selectively upregulated on accumulating IgG$^+$ B cells in natalizumab-treated MS blood (pre- versus 1-year post-Tx). Correspondingly, the abundance of CXCR3 on IgG1$^+$ B cells led to superior transmigration across human brain endothelial monolayers *in vitro*. Finally, CXCR3^{high}T-bet^{high} IgG1$^+$ B-cell frequencies correlated to those of IFN-γ^{high} Th17.1 and not Th17 cells in MS blood. This work demonstrates that T-bet is synergistically upregulated by IFNGR and TLR9 in B cells, probably underlying preferential CXCR3-mediated recruitment to the CNS and enhanced IgG1 responses to local antigens in MS.

BS.A.01.04
Extracellular Vesicles derived from licensed Mesenchymal Stem Cells: a tunable approach to regulate inflammatory angiogenesis

R. Angioni[1,2,3], *S. Herkenne*[1], *C. Liboni*[1,2], *R. Sanchez-Rodriguez*[1,2,3], *B. Cali*[3], *G. Borile*[3], *A. Viola*[3];
[1]*Padova University, Department of Biomedical Sciences, Via Ugo Bassi, 58/b, 35131- Padova, Italy, Padova, Italy,* [2]*VIMM- Venetian Institute of Molecular Medicine, Padova, Italy,* [3]*Fondazione Istituto di Ricerca Pediatrica Città della Speranza, Padova, Italy.*

Angiogenesis is the process that leads to the formation of new blood vessels from a pre-existing vascular network, playing a key role in many physiological and pathological processes. Consequently, targeting angiogenesis represents a very interesting therapeutic approach. We have already shown that mesenchymal stem cells (MSCs) stimulated with pro-inflammatory cytokines (st-MSCs) block angiogenesis through the release of soluble factors. Thus, we highlighted the endothelium as a novel target during the MSC immunosuppressive effect. However, the therapeutic employment of MSCs to control inflammation is far to be clinically translated. The development of a cell-free therapeutic approach could represent a better cost-effective and safer procedure. Here, we demonstrate that extracellular vesicles derived from stimulated MSC conditioned medium (EV stMSC-CM), but not from their unstimulated counterparts (EV unstMSC-CM), affect angiogenesis, thus recapitulating the MSC effect. EV stMSC-CM, expressing high levels of the ecto-5'-nucleotidase CD73, generate extracellular adenosine. We demonstrated that the EV stMSC-CM generated adenosine inhibits the endothelial cell migration, by inducing an excessive intracellular ROS accumulation, both *in vitro* and *in vivo*. These results indicate that EVs derived from st-MSCs display anti-angiogenic properties and could be exploited for cell-free therapeutic strategies. Additionally, they pave the way for a better understanding of the MSC physiological role *in vivo.*

BS.A.01.05
Lymph node stromal cell function upon immune activation is modulated by dynamics between podoplanin and its binding partners on the plasma membrane

C. M. de Winde*, A. C. Benjamin, L. Millward, V. G. Martinez, S. E. Acton;*
MRC Laboratory for Molecular Cell Biology, London, United Kingdom.

Lymph node expansion is a pivotal process during activation of an immune response, and is controlled by contractility through the fibroblastic reticular cell (FRC) network. Interactions between CLEC-2 expressed on dendritic cells (DCs) and podoplanin on FRCs results in rapid loss of actomyosin contractility and subsequent elongation of FRCs allowing lymph node expansion. Directly downstream, this interaction results in decreased membrane binding and phosphorylation of ezrin-radixin-moesin (pERM) proteins. We sought to understand the molecular mechanisms controlling podoplanin activity.
Podoplanin remains at the plasma membrane even when inhibited by CLEC-2. We hypothesized that podoplanin function is regulated by specific binding partners in different membrane compartments. Protein clustering within membranes is tightly controlled by specialized membrane structures, including lipid rafts and tetraspanin-enriched microdomains. Upon CLEC-2 binding, podoplanin preferentially clusters into cholesterol-rich membrane domains, containing CD44, a known podoplanin binding partner. We show a link between podoplanin and CD44 expression in FRCs. Knockdown of podoplanin coincides with decreased CD44 expression, and in contrast, CLEC-2 stimulation of FRCs increases CD44 expression. RNAseq analysis identified tetraspanins CD9 and CD82 as potential interaction partners of podoplanin. Similarly to CD44, expression of both CD9 and CD82 was altered by contractility, podoplanin knockdown and CLEC-2 stimulation.
Our data support a model whereby podoplanin on FRCs resides in tetraspanin-enriched microdomains, and that CLEC-2 binding may orchestrate a switch between active and inactive complexes. This molecular switch allows for rapid yet reversible inhibition of contractility in FRCs, allowing the lymph node to undergo continuous cycles of remodelling during immune responses.

BS.A.01.06

Autoimmune associated gene PTPN22 is a novel negative regulator of dendritic cell homeostasis and function

H. A. Purvis[1], *F. Clarke*[1], *G. H. Cornish*[1], *T. J. Peel*[1], *C. Sanchez-Blanco*[1], *D. J. Rawlings*[2], *R. Zamoyska*[3], *P. Guermonprez*[1], *A. P. Cope*[1];
[1]*CMCBI Kings College London, London, United Kingdom,* [2]*University of Washington School of Medicine, Seattle, United States,* [3]*Institute of Immunology and Infection Research, Edinburgh, United Kingdom.*

Classical CD11c$^+$MHCcII$^+$ dendritic cells (DC) are divided into functionally distinct phenotypes including the SIRPα$^+$ DC2 subset. Splenic DC2 activate CD4$^+$ T-cells and are potent inducers of follicular helper T-cells (T_{FH}) stimulating germinal center (GC) formation. A single nucleotide polymorphism within the phosphatase *PTPN22* confers an enhanced risk of developing multiple autoimmune diseases including rheumatoid arthritis and type 1 diabetes. PTPN22 negatively regulates multiple immunoreceptor signalling cascades and *Ptpn22*$^{-/-}$ mice display an expanded T_{FH} repertoire and increased GCs. We observed a specific expansion in the number and proportion of splenic ESAM^{HI} DC2 within *Ptpn22*$^{-/-}$ and *Ptpn22*^{R619W} (orthologue of the human autoimmune associated variant) mice compared to WT. Competitive adoptive bone marrow transfers revealed that PTPN22 negatively regulates DC2 development in a DC intrinsic manner. PTPN22-mediated DC2 expansion occurred post-DC precursor development and was not conferred by enhanced responsiveness to Flt3L or GM-CSF. Our data indicate that PTPN22 regulates lymphotoxin β receptor responsiveness to expand ESAM^{HI} DC2. To assess if expanded DC2 were capable of mediating enhanced T_{FH}, CD4$^+$ OT-II T-cells were co-cultured with splenic DCs derived from WT and *Ptpn22*$^{-/-}$ mice immunised with ovalbumin targeted to DC2 via receptor DCIR2 (anti-DCIR2-ova) and sheep RBC adjuvant. Interestingly, the expansion of DC2, conferred by *Ptpn22*$^{-/-}$, was sufficient to induce enhanced T_{FH} responses, and therefore may contribute to the expanded T_{FH} and GCs observed in Ptpn22 variant mice *in vivo*. Together these data provide a new insight into how perturbations to PTPN22 contributes to generate pathogenic autoimmune responses by altering DC2 homeostasis and function.

BS.B.01 Bright Sparks B

BS.B.01.01

Immunotargeting of acute myeloid leukemia by highly polyclonal Delta One T cells

B. Di Lorenzo[1], *A. E. Simões*[2], *F. Caiado*[1], *P. Tieppo*[3], *D. V. Correia*[1], *J. Déchanet-Merville*[4], *T. Schumacher*[5], *H. Norell*[1], *I. Prinz*[6], *S. Ravens*[6], *D. Vermijlen*[3], ***B. Silva-Santos***[1];
[1]*Instituto de Medicina Molecular, Lisboa, Portugal,* [2]*Lymphact S.A., Coimbra, Portugal,* [3]*Université Libre de Bruxelles, Brussels, Belgium,* [4]*Université de Bordeaux, Bordeaux, France,* [5]*Netherlands Cancer Institute, Amsterdam, Netherlands,* [6]*Hannover Medical School, Hannover, Germany.*

The adoptive transfer of CAR-T cells has transformed the treatment of B-cell acute lymphoblastic leukemia, but acute myeloid leukemia (AML), the deadliest of all hematological cancers, remains a major challenge due to absence of a targetable tumor-specific antigen. We are considering the potential of Vdelta1+ gamma-delta T cells, which are known to associate with improved survival of transplanted patients, but have never been applied as adoptive cell therapy. Having recently developed a clinical-grade protocol for expansion and differentiation of cytotoxic Vdelta1+ T cells, termed "Delta One T" (DOT) cells, we characterized here their anti-AML reactivity on the path to therapeutic application. DOT cells were highly cytotoxic against a large panel of AML primary samples and cell lines, including cells selected for resistance to standard chemotherapy. Interestingly, DOT cell targeting did not select for particular AML lineages, suggesting a very broad recognition domain. Indeed, next-generation sequencing of the CDR3 regions in *TRGV* and *TRDV* genes showed a striking polyclonality of DOT cells compared to ex vivo Vdelta1+ T cells, notably approaching that of naïve Vdelta1+ thymocytes. By establishing (single cell-derived) DOT cell clones we demonstrate that the vast majority display potent anti-AML cytotoxicity, which at bulk level translates into efficient AML targeting in vitro and in vivo. In particular, the adoptive transfer of DOT cells markedly reduced AML load in the blood and target organs of human AML xenografts; and significantly prolonged host survival. This works thus provides the pre-clinical proof-of-concept for application of highly polyclonal DOT cells in AML treatment.

BS.B.01.02

Broad tumor microenvironment remodelling after oncolytic Adenovirus coding for TNFa and IL-2 enable T-cell therapies to deliver complete responses in solid tumors

V. Cervera-Carrascon[1,2], *R. Havunen*[1,2], *J. M. Santos*[1,2], *M. Siurala*[1,2], *S. Sorsa*[1,2], *A. Hemminki*[1,2,3];
[1]*TILT Biotherapeutics, Helsinki, Finland,* [2]*University of Helsinki, Helsinki, Finland,* [3]*Comprehensive Cancer Center, Helsinki Universitary Hospital, Helsinki, Finland.*

Besides the considerable potential of T-cell therapies for the treatment of cancer, their clinical outcome is unimpressive on solid tumours. One likely limitation here is the tremendous complexity of the tumour microenvironment, which render inefficient the treatments through multiple suppressive mechanisms. An oncolytic adenovirus engineered to express tumour necrosis factor alpha and interleukin-2 (Ad5/3-E2F-d24-hTNFa-IRES-hIL2, a.k.a. TILT-123) was empirically designed to tackle tumour immune suppression and enable T-cell therapies against solid tumours. A subcutaneous pancreatic tumour model in Syrian hamsters provided the means to study the antitumor efficacy of TILT-123 in combination with tumour infiltrating lymphocyte (TIL) transfer since it supports adenoviral replication in addition of the immune competency of the animals. More studies were performed focusing on safety parameters, abscopal effect, trafficking of the TILs transferred towards the tumour or the fact that TILT-123 can replace lympho-depleting preconditioning and postconditioning with recombinant interleukin-2 systemically. Other studies were carried out in a subcutaneous melanoma model (B16.OVA) in mice (with a murine version of the viruses) exploring other T-cell therapies such as adoptive cell transfer of T cells with OVA-specific TCR or even checkpoint blockade. From those studies, all the animals got complete responses when treated with adoptive cell transfer or checkpoint blockade, combined with the viral therapies. Analysing the biological samples from those experiments revealed a broad tumour microenvironment remodelling, including higher presence of CD8+ lymphocytes, NK cells and mature DCs, while a decreased M2 macrophage manifestation. Based on these results a Phase-I clinical trial will start in 2019.

BS.B.01.03

NF-kB c-Rel is crucial for the regulatory T cell immune checkpoint in cancer

Y. Grinberg-Bleyer[1,2], *H. Oh*[2], *A. Desrichard*[3], *T. Chan*[3], *R. Schmid*[4], *M. Hayden*[2], *U. Klein*[5], *S. Ghosh*[2];
[1]*Centre de Recherche en Cancerologie de Lyon, Lyon, France,* [2]*Columbia University Medical Center, New York, United States,* [3]*Memorial Sloan Kettering Cancer Center, New York, United States,* [4]*Technische Universität Munich, Munich, Germany,* [5]*University of Leeds, University of Leeds, United Kingdom.*

Cancers use a wide variety of mechanisms to dampen tumor immune response. Among them, CD4+Foxp3+ regulatory T cells (Tregs) are largely described to inhibit the function of effector cells. Therefore, precisely understanding the mechanisms governing Treg homeostasis may be a valuable strategy to enhance immune responses against cancer. Here we have examined the roles of the p65 and c-Rel subunits of the NF-kB family of transcription factors in Treg biology. We found that specific ablation of one or both NF-kB subunits in Tregs drove a gradual autoimmune syndrome. This was associated with profound changes in the molecular signature of NF-kB deficient Tregs. Remarkably, c-Rel specifically maintained gene expression pattern in activated Tregs, which are potent inhibitors of anti-tumor immunity. This led us to explore the role of NF-kB in Treg homeostasis during tumor growth. Strikingly, melanoma growth was drastically reduced in mice lacking c-Rel, but not p65, in Tregs. This was associated with an increased effector T cell activation. Moreover, chemical inhibition of c-Rel delayed melanoma growth and potentiated anti-PD-1 checkpoint-blockade therapy by impairing the Treg transcriptional program. Our data demonstrate a specific role for each NF-kB subunit in Treg function and homeostasis, and highlights a new therapeutic opportunity for the treatment of cancer. *Reference: Grinberg-Bleyer et al, "NF-kB c-Rel is crucial for the regulatory T cell immune checkpoint in cancer."* ***Cell****, 2017 Sep 7;170(6):1096-1108*

BS.B.01.04

Mitochondrial DNA shapes metabolism in immune response

A. V. Lechuga-Vieco[1,2], *G. Prota*[3], *U. Gileadi*[3], *A. Latorre-Pellicer*[1], *J. Pellico*[1], *J. Ruíz-Cabello*[4,5,2], *V. Cerundolo*[3], *J. A. Enriquez*[1,6];
[1]*Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain,* [2]*Centro de Investigación en Red de Enfermedades Respiratorias (CIBERES), Madrid, Spain,* [3]*MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom,* [4]*CIC biomaGUNE, San Sebastián-Donostia, Spain,* [5]*Universidad Complutense, Madrid, Spain,* [6]*Centro de Investigación Biomédica en Red Fragilidad y Envejecimiento Saludable (CIBERFES), Madrid, Spain.*

All mitochondrial DNAs (mtDNA) of a given cell in our organism are essentially identical, a situation termed homoplasmy. Animal models with identical nuclear genomes but with different mtDNA haplotypes (conplastic mice) generate functionally different OXPHOS systems that shape the organismal metabolism[1], supporting the conclusion that different mtDNA haplotypes are phenotypically relevant.
There is an unsolved controversy regarding the possible functional consequences of different physiological haplotypes of mtDNA in inflammatory processes. To address this issue, we have characterised conplastic mice throughout their lifespan through transcriptomic, metabolomic, biochemical and phenotypical studies. We have also applied *in vivo* imaging techniques for non-invasive assessment of tissue metabolism and inflammatory processes. We find that the mtDNA haplotype profoundly influences reactive oxygen species (ROS) generation, energy homeostasis metabolism, immune response and ageing among others, resulting in different healthy longevities of conplastic and heteroplasmic strains. Elucidating the role of mitochondrial genetics in immune cell metabolism and function combined with an understanding of the mechanisms regulating this process is quite challenging. To address these questions, we engineered heteroplasmic mice, with two wild-type mtDNA variants co-existing in the same cytoplasm, which provided an opportunity to demonstrate mtDNA segregation in T cells, upon their in vivo and in vitro activation. In addition, we observed how differences in OXPHOS performance and ROS production can modulate T cell function and differentiation. MtDNA haplotype profoundly impacts on the way immune cells respond to and influence their environment, ultimately shaping immune cell function and fate.

[1] Latorre-Pellicer, A. *et al*. (2016). Nature 535, 561–565

BS.B.01.05

***Milieu Interieur*: defining immunological variability through systems biology for a better understanding of disease**

D. Duffy[1], *E. Patin*[1], *C. Posseme*[1], *B. Charbit*[1], *J. Bergsted*[2], *H. Quach*[1], *V. Rouilly*[1], *M. Hasan*[1], *J. Fellay*[3], *J. Di Santo*[1], *M. Albert*[4], *L. Quintana Murci*[1], *Milieu Intérieur Consortium;*
[1]*Institut Pasteur, Paris, France,* [2]*Lund University, Lund, Sweden,* [3]*EPFL, Lausanne, Switzerland,* [4]*Genentech, San Francisco, United States.*

Immune responses are highly variable between individuals and populations, with this variance driven by genetic and environmental factors. To better define this inherent variability and to dissect its causes the *Milieu Interieur* cohort was established consisting of 1,000 healthy donors stratified by age (20-70 years old) and sex (50:50). From these donors we have characterized baseline immune phenotypes by multi-parameter flow cytometry, and induced immune responses in standardized whole blood stimulations systems. We have previously shown how innate immune cells are preferentially shaped by genetics, while adaptive immune cells are more impacted by environmental factors. More recently we have defined key immune stimuli that capture the full breadth of induced immune responses and characterized these responses at transcriptional and proteomic levels. We are currently integrating this rich data set with complementary cellular, genetic and epigenetic data sets in a systems immunology approach. Classical statistical approaches as well as machine learning techniques are being applied to define immune response networks and determine which components of these pathways are controlled at the genetic level. In parallel disease specific immune perturbations in infection and autoimmunity have been identified utilizing the same standardized approach. Direct comparison with our well defined 1,000 healthy donor cohort is allowing for a new understanding of these mechanisms in disease. In summary, these results will lay the foundations for the integration of immune response variability in smart clinical study design and eventually precision medicine strategies.

BS.B.01.06

T cell - target cell communication is determined by glycosphingolipid-mediated shielding of cell surface proteins

A. A. de Waard[1], *M. L. Jongsma*[1], *T. Zhang*[2], *S. Holst*[2], *M. Wuhrer*[2], *C. E. van der Schoot*[1], *R. M. Spaapen*[1];
[1]*Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands,* [2]*Leiden University Medical Center, Leiden, Netherlands.*

Receptor-ligand interactions are essential for immune cell function. Using state-of-the-art genome-wide screens we identified that physical accessibility of surface MHC class I (MHC-I) can be restricted by a subtype of glycosphingolipids (GSLs), so-called (neo-)lactoseries GSLs. Moreover, tumor cells expressing these GSLs have a reduced capacity to activate CD8+ T cells. Here, we hypothesized that also cell surface proteins other than MHC-I are shielded by GSLs preventing the binding of their natural receptors.
Using CRISPR/Cas9 we generated various cell lines with a different GSL composition as confirmed using mass spectrometry. These cells and control cells were fluorescently barcoded to analyze in a single well by flow cytometry. We then screened the various cell lines for new cell surface proteins with a restricted physical accessibility, just like MHC-I, using a custom monoclonal antibody array. Out of 26 proteins analyzed, the accessibility of 18 cell surface proteins was specifically reduced by the (neo-)lactoseries GSL subtype. Although GSLs are described to be enriched in specific membrane microdomains, both proteins within and outside of these microdomains were affected by GSLs. Importantly, next to restricting accessibility of surface proteins for antibodies, the (neo-)lactoseries GSLs largely blocked the ligation of the receptors LIR-1 and SIRP-α to their ligands, MHC-I and CD47 respectively.
To conclude, the GSL repertoire regulates shielding of several surface proteins which affects the interaction with CD8+ T cells. Tumors and viruses may thus specifically corrupt intercellular communication with the immune system through alterations in the cellular GSL signature.

BS.C.01 Bright Sparks C

BS.C.01.01

Tissue resident memory T-cell (T_{RM}) maintenance is regulated by tissue damage

R. Stark[1,2], *T. H. Wesselink*[1], *F. M. Behr*[1,2], *N. A. Kragten*[1], *K. P. van Gisbergen*[1,2], *R. A. van Lier*[1,2];
[1]*Sanquin Research, Amsterdam, Netherlands,* [2]*Academic Medical Center, Amsterdam, Netherlands.*

Tissue resident memory T cells (T_{RM}) are non-circulating immune cells that contribute to the first line of local defense against reinfections. Their location at hot spots of pathogen encounter frequently exposes T_{RM} to tissue damage. This history of danger-signal exposure is an important aspect of T_{RM} mediated immunity that has been overlooked so far. RNA-sequencing revealed that T_{RM} from liver and small intestine express P2RX7, a damage/danger associated molecular pattern (DAMP)-receptor that is triggered by extracellular nucleotides (ATP, NAD). We confirmed that P2RX7 protein was expressed in CD8 T_{RM} but not circulating cells (T_{CIRC}) across different infection models. Tissue damage induced during the routine liver preparation led to P2RX7 activation and resulted in selective cell death of T_{RM} but not T_{CIRC}. P2RX7 activation *in vivo* by exogenous NAD led to a specific depletion of T_{RM} while retaining T_{CIRC}. The effect was absent in P2RX7 deficient mice and after P2RX7-blockade. TCR-triggering down-regulated P2RX7 expression and made T_{RM} resistant to NAD-induced cell death. Physiological triggering of P2RX7 by sterile tissue damage during drug-induced liver injury lead to a loss of previously acquired pathogen-specific liver T_{RM} in WT but not P2RX7KO T cells of mixed bone marrow chimeras.
Our results highlight P2RX7-mediated signaling as a novel pathway for the regulation of T_{RM} maintenance. Extracellular nucleotides released during infection and tissue damage could deplete T_{RM} locally and free niches for new and infection-relevant specificities. This suggests that the recognition of tissue damage promotes persistence of antigen-specific over bystander T_{RM} in the tissue niche.

BS.C.01.02

***TLR7* escapes X chromosome inactivation in immune cells**

J. Guéry;
INSERM U1043, CPTP, Toulouse, France.

Toll-like receptor 7 (TLR7), an endosomal sensor of exogenous and autologous single-stranded RNA, is critical to the induction of antiviral immunity. TLR7 is also a pathogenic factor in systemic lupus erythematosus (SLE), where it senses RNA-containing immune complexes. Disease severity in lupus-prone mice depends on *Tlr7*. Like other autoimmune disorders, SLE is strongly sex-biased, and female-to-male incidence peaks at 9:1 in adulthood. However, men with Klinefelter syndrome (47,XXY) carry a similar risk of SLE compared to women, suggesting a gene-dose effect of X chromosome loci. *TLR7* localizes to Xp outside the pseudoautosomal regions and we investigated whether TLR7 gene might escape X-inactivation in primary immune cells of women, such as B cells, pDCs and monocytes. Our results show that *TLR7* escapes X chromosome inactivation in distinct populations of pDCs, B cells and monocytes from women and 47, XXY men. Cells exhibiting bi-allelic expression of *TLR7* were observed in all donor tested, with frequencies ranging from 7-45%. Using RNA-FISH, we show the presence of primary *TLR7* transcripts on both X chromosomes in a subset of B lymphocytes and pDCs from women. Focusing on women's B lymphocytes, we show that bi-allelism is associated with increased gene dosage and imparts a selective advantage at key TLR-dependent development checkpoints of effector B cells. Taken together, our work show that *TLR7* bi-allelism defines a new women-specific functional subset of B cells, and mechanistically connects TLR7-driven B-cell responses to sex bias in SLE and other autoimmune disorders. (Souyris et al., ***Sci. Immunol.*** 2018 3:eaap8855).

BS.C.01.03

Human regulatory T cells suppress CD4+ T cells by rapidly altering the phosphoproteome

R. N. Joshi[1], *F. Marabita*[1], *N. Binai*[2], *Z. Sui*[3], *A. Altman*[3], *A. J. Heck*[2], *J. Tegner*[1], *A. Schmidt*[1];
[1]*Karolinska Institute, Stocholm, Sweden,* [2]*Utrecht University, Utrecht, Netherlands,* [3]*La Jolla Inst. for Allergy and Immunology, La jolla, United States.*

Regulatory T cells (Tregs) control key events of immunity primarily by suppression of effector T cells, and Treg dysfunction is involved in the pathogenesis of autoimmunity, allergy and cancer. We previously revealed that Tregs rapidly suppress T cell receptor (TCR)-induced calcium store depletion in conventional CD4+CD25− T cells (Tcons) independently of IP3 levels, consequently inhibiting NFAT signaling and effector cytokine expression.
Here, we study Treg suppression mechanisms through unbiased phosphoproteomics of primary human Tcons upon TCR stimulation and Treg-mediated suppression, respectively. We have demonstrated that Tregs suppress the signalling cascade in Tcons by inducing a state of overall decreased phosphorylation as opposed to TCR stimulation. Further we also discovered novel phosphosites (T595_S597) in the DEF6 (SLAT) protein that were phosphorylated upon TCR stimulation and conversely dephosphorylated upon coculture with Tregs. Mutation of these DEF6 phosphosites abrogated interaction of DEF6 with the IP3 receptor and affected NFAT activation and cytokine transcription in primary Tcons. Additionally, we also discovered a phosphatase inhibitor from our phosphoproteomic screen, the loss of which rendered the Tcons to be resistant to Treg-mediated suppression. These novel mechanisms and phosphoproteomics data resource may aid in modifying sensitivity of Tcons to Treg-mediated suppression and contribute to improve treatment of autoimmunity and cancer, particularly considering the frequently observed resistance of target Tcons to Treg-mediated suppression in human autoimmune disease.

BS.C.01.04

Prophylactic treatment with novel forms of allergen-laden virus like-nanoparticles (VNP) induces specific tolerance in a mouse model of allergy

B. Kratzer[1], *C. Köhler*[1], *S. Hofer*[1], *U. Smole*[1], *D. Trapin*[1], *J. Iturri*[2], *D. Pum*[2], *P. Kienzl*[3], *A. Elbe-Bürger*[3], *P. Gattinger*[4], *I. Mittermann*[4], *B. Linhart*[4], *Y. Dorofeeva*[4], *B. Jahn-Schmid*[4], *A. Neunkirchner*[1], *R. Valenta*[4], *W. F. Pickl*[1];
[1]*Institute of Immunology; Center for Pathophysiology, Infectiology and Immunology; Medical University, Vienna, Austria,* [2]*Department of Nanobiotechnology, Institute for Biophysics, University of Natural Resources and Life Sciences, Vienna, Austria,* [3]*Department of Dermatology, Laboratory of Cellular and Molecular Immunobiology of the Skin, Medical University, Vienna, Austria,* [4]*Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University, Vienna, Austria.*

In high-risk populations, allergen-specific prophylaxis could protect from sensitization and subsequent development of allergic diseases. However, such treatment might itself induce sensitization and allergies. Therefore, new non-allergenic vaccine formulations, such as virus like nanoparticles (VNP) are needed. We here targeted the major mugwort allergen Art v 1 either to the surface or to the inner side of VNP by genetic engineering and subjected the vaccine candidates to biochemical and immunological analyses *in vitro,* as well as in a humanized mouse model of mugwort allergy *in vivo*. Degranulation of RBL cells sensitized with Art v 1-specific IgE occurred exclusively upon incubation to VNP expressing surface-exposed but not shielded allergen, whereas both VNP versions induced proliferation and cytokine production of allergen-specific T cells *in vitro.* Upon intranasal application in mice, VNP expressing surface-exposed allergen induced allergen-specific antibodies, including IgE, which was not observed for VNP expressing shielded allergen, making them promising candidates for prophylactic application. Preventive treatment with VNP expressing shielded allergen protected mice from subsequent sensitization with mugwort pollen extract. Protection was associated with a Th1/Treg-dominated cytokine response, increased Foxp3$^+$ Treg numbers in lungs and reduced lung resistance. *In vivo,* fluorescently labeled VNPs were predominantly taken up by alveolar macrophages but also CD103$^+$ DCs, which are known to be strong inducers of Foxp3$^+$ Treg. Allergen-laden VNPs represent a novel and versatile *in vivo* allergen delivery platform to selectively target T cells that can be used for immunotherapy without inducing de novo sensitization. *Supported by Austrian Science Fund (FWF) DK-W1248, SFB-F4609, F4605.*

BS.C.01.05

Macrophages require CD200 receptor to resolve inflammatory pain

R. Raoof*, M. van der Vlist, H. Willemen, J. Prado Sanchez, L. Meyaard, N. Eijkelkamp;*
University Medical Center Utrecht, Utrecht, Netherlands.

Pain is a cardinal symptom of inflammation. The resolution of pain is presumed to be the result of discontinued inflammatory processes, yet in various diseases such as rheumatic diseases pain persists even when inflammation has subsided. Here we investigated the contribution of macrophages in the regulation of inflammatory pain. Transient inflammatory pain was induced by injection of carrageenan in mouse hind paws. In this model, monocytes/macrophages infiltrated the dorsal root ganglia (DRG) that are distant from inflammation and contain the cell bodies of sensory neurons innervating the hind paw. Unexpectedly, monocyte/macrophage-depleted mice failed to resolve inflammatory pain whilst the duration of carrageenan-induced inflammation was unaffected. During transient pain, DRG-infiltrating macrophages expressed the M2 marker CD206, but not M1 marker iNOS. Intrathecal injection of CD115$^+$ monocytes, M0 macrophages, or *in-vitro* M2-polarized macrophages in monocyte/macrophage depleted mice resolved inflammatory pain, whilst M1 macrophages were unable to resolve pain. M2 Macrophages express high levels of the immune inhibitory CD200-Receptor (CD200R). Similar to macrophage depleted mice, CD200R$^{-/-}$ mice failed to resolve inflammatory pain. Resolution of inflammatory pain required CD200R expression on monocytes: in monocyte/macrophage depleted mice, administration of WT, but not CD200R$^{-/-}$ monocytes, rescued resolution of pain. Moreover, injection of WT monocytes in CD200R$^{-/-}$ mice restored the capacity to resolve inflammatory pain.
In conclusion, we show that monocytes/macrophages require CD200R to drive the resolution of inflammatory pain in the DRG, and thereby prevent development of chronic pain. These data indicate that monocyte/macrophage function extends beyond control of inflammation to the regulation of pain-sensing neurons.

BS.C.01.06

Tissue-resident memory CD8$^+$ T cells form systemic effector and memory responses but 'remember' their site of origin

F. M. Behr*, T. H. Wesselink, L. Parga Vidal, N. A. Kragten, R. Stark, K. P. van Gisbergen;*
Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands.

Tissue-resident memory CD8$^+$ T cells (T_{RM}) are non-circulating memory T cells localizing to peripheral (barrier) tissues. T_{RM} provide efficient early protection against local reinfection through rapid cytokine production and local proliferation. However, the contribution of T_{RM} to systemic secondary effector and memory responses remains unclear.
In order to investigate T_{RM} responses after re-challenge *in vivo*, we established an adoptive transfer model of memory CD8+ T cells arising after acute viral infection. Intestinal T_{RM} and circulating effector memory (T_{EM}) and central memory CD8$^+$ T cells (T_{CM}) were isolated from immune mice, and co-transferred into naïve recipients. Unlike their circulating counterparts, T_{RM} cells were confined to non-lymphoid peripheral tissues following transfer and retained a resident phenotype. After viral challenge, T_{RM} cells, similar to circulating memory subsets, gave rise to a systemic recall response, albeit of reduced magnitude. Upon viral clearance, T_{RM} formed circulating and resident secondary memory cells, but did not give rise to T_{CM} cells. Interestingly, the intestine-derived T_{RM} were superior at re-generating secondary T_{RM} in the intestinal compartment, but not at other sites. This was consistent with their selective re-expression of the intestine-homing receptor CCR9. In contrast, re-activated liver T_{RM} lacked the capacity to access the intestine, preferentially accumulated in the liver and upregulated receptors associated with liver-homing.
Our findings demonstrate that T_{RM} have the potential to generate body-wide effector and memory responses, but retain an intrinsic preference for their tissue of origin. This may pose important implications on future cell therapy and vaccination strategies that employ T_{RM}.

BS.D.01 Bright Sparks D

BS.D.01.01

Intestinal IgA shows specific broad binding to phylogenetically non-related bacteria

J. Kabbert[1], *H. Wardemann*[2], *O. Pabst*[1];
[1]*Uniklinik RWTH Aachen, Institute of Molecular Medicine, Aachen, Germany,* [2]*German Cancer Research Center, Division of B cell Immunology, Heidelberg, Germany.*

Secretory immunoglobulin A (SIgA) is a key component in gut homeostasis. SIgA binds to luminal and gut epithelial surface associated bacteria, thereby contributing to intestinal host defense against pathogens and maintaining a stable microbiota composition. Considering the vast changes in the microbiota consortium depending on diet, medical treatment and infection, it remains elusive how the host immune system can generate and regulate beneficial SIgA responses to a highly dynamic commensal setting.
This study sought to profile the binding spectrum of monoclonal IgA antibodies (mABs) derived from human healthy donors and inflammatory bowel disease (IBD) patients by flow cytometry. Screening of almost 200 monoclonal IgA antibodies revealed an unexpected high frequency of mABs with substantial microbiota reactivity. In order to determine the binding spectrum of microbiota reactive mABs, we used these mABs to stain bacteria isolated from RAG$^{-/-}$ feces with subsequent 16S rDNA sequencing of bound and unbound bacterial fractions. Individual mABs bound a diverse spectrum of commensals rather than showing reactivity to single taxa. This suggests that single monoclonal mABs functionally bind a relevant fraction of different intestinal bacteria *in vivo*. Unlike recent reports, we did not observe a correlation of high microbiota reactivity and polyreactivity of respective mABs. In addition, while mABs with microbiota reactivity showed frequent somatic mutations, germ-line variants of previously high binding mABs showed decreased microbiota binding or an altered binding profile. We therefore speculate that ongoing somatic hypermutation selects for intestinal IgA with broad, yet specific binding to different bacterial taxa.

BS.D.01.02

Oxysterol sensing through the receptor GPR183 promotes the lymphoid tissue-inducing function of innate lymphoid cells and colitis

J. Emgård[1], *H. Kammoun*[1], *B. García-Cassani*[2], *J. Chesné*[2], *S. Parigi*[1], *J. Jacob*[3], *H. Cheng*[4], *E. Evren*[1], *S. Das*[1], *P. Czarnewski*[1], *N. Sleiers*[1], *F. Melo-Gonzalez*[5], *E. Kvedaraite*[1], *M. Svensson*[1], *E. Scandella*[4], *M. Hepworth*[5], *S. Huber*[6], *B. Ludewig*[4], *L. Peduto*[3], *E. Villablanca*[1], *H. Veiga-Fernandes*[2], *J. Pereira*[7], *R. Flavell*[7], ***T. Willinger***[1];
[1]*Karolinska Institutet, Stockholm, Sweden,* [2]*Champalimaud Centre For The Unknown, Lisbon, Portugal,* [3]*Institut Pasteur, Paris, France,* [4]*Institute of Immunobiology, St. Gallen, Switzerland,* [5]*University of Manchester, Manchester, United Kingdom,* [6]*University Hospital Eppendorf, Hamburg, Germany,* [7]*Yale University, New Haven, United States.*

Innate lymphoid cells (ILCs) sense environmental signals and are critical for healthy organ function in the intestine. Yet, which signals are sensed and what receptors control ILC function remains poorly understood. Here we demonstrate that group 3 ILCs (ILC3s) sense hydroxylated metabolites of cholesterol (oxysterols) through the G protein-coupled receptor (GPR183), which was highly expressed by ILC3s with a lymphoid tissue-inducer phenotype. GPR183 and its oxysterol ligand 7α,25-hydroxycholesterol (7α,25-OHC) directed the migration of ILC3s to lymphoid structures in the colon and ablation of the *Gpr183* gene in ILC3s caused a defect in the formation of colonic cryptopatches and isolated lymphoid follicles. The same phenotype was observed in mice lacking *Ch25h*, demonstrating a requirement for oxysterols in lymphoid tissue organogenesis. Fibroblastic stromal cells found in intestinal lymphoid structures expressed 7α,25-OHC-synthesizing enzymes and provided the local source of oxysterols. In contrast, GPR183 and its ligand 7α,25-OHC were dispensable for lymphoid tissue formation in the small intestine. Furthermore, 7α,25-OHC was increased by inflammatory signals and GPR183 controlled inflammatory cell recruitment during colitis. Consequently, *Gpr183*-deficient mice were less susceptible to colitis in an innate model of intestinal inflammation. Our results identify oxysterols as a novel class of molecules that control ILC function and establish an unexpected link between cholesterol metabolism, ILC3 migration, lymphoid tissue development, and intestinal homeostasis.

BS.D.01.03

Eomes broadens the scope of CD8 T cell memory by inhibiting apoptosis in low-affinity cells

I. Kavazović[1], *E. Slinger*[2], *G. Balzaretti*[3], *P. Klarenbeek*[3], *H. Han*[4], *N. A. Lemmermann*[5], *A. ten Brinke*[6], *J. Koster*[7], *S. Jonjić*[1], *N. de Vries*[3], *Y. Bryceson*[4], *B. Polić*[1], *E. Eldering*[2], *F. M. Wensveen*[1];

[1]*School of Medicine, University of Rijeka, Rijeka, Croatia,* [2]*Dept. of Experimental Immunology, AMC Amsterdam, Amsterdam, Netherlands,* [3]*Dept. of Clinical Immunology & Rheumatology, AMC Amsterdam, Amsterdam, Netherlands,* [4]*Center for Hematology and Regenerative Medicine, Karolinska University Hospital Huddinge, Stockholm, Sweden,* [5]*Institute for Virology and Research Center for Immunotherapy (FZI) at the University Medical Center of the Johannes Gutenberg University, Mainz, Germany,* [6]*Dept. of Immunopathology, Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands,* [7]*Dept. of Oncogenomics, AMC Amsterdam, Amsterdam, Netherlands.*

The memory CD8 T cell pool must select for high-affinity clones to efficiently counter re-infection, yet must retain a level of clonal diversity to allow recognition of pathogens with mutated immuno-dominant epitopes. How this is mediated is unclear, especially in the context of a selective drive for antigen-affinity. We find that two distinct mechanisms of memory cell selection operate. Using an inducible system in which we can time elimination of Eomes by Poly(I:C) injection we show that low-affinity memory exclusively depends on the transcription factor Eomes in the first days after antigen encounter. Eomes is induced at low activating signal strength and directly drives transcription of the pro-survival protein Bcl-2. At higher signal intensity T-bet is induced which suppresses Bcl-2, generating a survival advantage for low-affinity cells. In contrast, high-affinity cells form memory independent of Eomes, but have a proliferative advantage over low-affinity cells, which compensates for their survival deficit. The Eomes-deficient CD8 T cell memory population therefore lacks low-affinity cells, resulting in a strongly reduced capacity to target antigen with point mutations in its immuno-dominant epitope. By specifically targeting Bcl-2 with a small molecule inhibitor early during the immune response we could increase the specificity of the CD8 T cell response. In summary, we demonstrate on a molecular level how sufficient diversity of the memory pool is established in an environment of affinity-based selection. Moreover, we demonstrate that the Eomes/Bcl2 axis may be exploited therapeutically to modify the scope of T cell based vaccines.

BS.D.01.04

Pentraxin3 Regulates IL-17A Mediated Immunity to *Leishmania*

G. Gupta*, P. Jia, J. E. Uzonna;*
University of Manitoba, Winnipeg, Canada.

Cutaneous leishmaniasis (CL), caused by the protozoan parasite *Leishmania (L) major*, results in ulcerative skin lesions at the sites of infection. Studies show that the nature of immune response plays a crucial role in resolution of skin lesions during infection. The long Pentraxin 3 (PTX3), a soluble pattern recognition molecule, is critical for wound healing by regulating tissue repair and innate and adaptive responses during infection and inflammation. Here, we show that PTX3 contributes to susceptibility to CL. PTX3$^{-/-}$ mice were highly resistant to primary and secondary *L. major* infections. Interestingly, the enhanced resistance of PTX3$^{-/-}$ mice to *L. major* was not associated with enhanced IFN-γ or decreased IL-4 response. Instead, L. *major*-infected PTX3$^{-/-}$ mice displayed strong IL-17 response and *in vivo* neutralization of IL-17A abolished their enhanced resistance and resulted in elevated parasite burden compared to their untreated controls. In *in vitro* polarization studies, more naïve CD4^{+} T cells from PTX3$^{-/-}$ mice significantly differentiated into Th17 cells compared to those from WT mice. This was associated with increased expression of Th17-specific transcription factors like RORγt and STAT3. Addition of recombinant PTX3 into Th17 polarizing cultures of PTX3$^{-/-}$ CD4^{+} T cells led to significant reduction in the expression of Th17-specific transcription and the frequency of Th17 cells. Collectively, our results show that PTX3 contributes to pathogenesis of CL by negatively regulating inflammation via enhancing IL-17 response.

BS.D.01.05

Imaging the host-pathogen interaction in tuberculosis in a bioelectrospray 3D cell culture model

E. Konstantinopoulou[1], *L. B. Tezera*[1], *S. Mahajan*[2], *P. T. Elkington*[1];
[1]*University of Southampton, Faculty of Medicine, Southampton, United Kingdom,* [2]*University of Southampton, Chemistry, Southampton, United Kingdom.*

Introduction: Tuberculosis is a deadly infectious disease caused by the bacterium *Mycobacterium tuberculosis* (*Mtb*). Traditional animal models as well as conventional 'two-dimensional' cell cultures do not accurately mimic human tuberculosis infection *in vivo*, such as the formation of caseating granulomas and degradation of extracellular matrix. Materials and Methods: We study a bioelectrospray-generated 3D cell culture model of tuberculosis, using diverse imaging techniques. In the 3D culture, *Mtb*-infected PBMCs (strains: H37Rv, 0414B) are mixed with an alginate-collagen gel. We investigated the host-pathogen interaction at high resolution at various stages of infection using Transmission Electron Microscopy (TEM). We used Micro-Computed Tomography (μCT) to show distribution of PBMCs in 3D in comparison to a human tuberculous lung biopsy. This was then correlated to traditional H&E, and matrix staining of the sectioned lung block. Using label-free microscopy [Coherent Anti-Stokes Raman Scattering (CARS), Second Harmonic Generation (SHG)], PBMC aggregation and collagen fibers were imaged.
Results: More lipid bodies were detected by TEM in the *Mtb*-infected samples than the uninfected controls. TEM and SHG imaging revealed collagen fibres attached to the surface of PBMCs. CARS microscopy showed that infection with *Mtb,* as well as the presence of collagen in the 3D matrix, influence the number of PBMC aggregates forming within the 3D culture. Preliminary data from μCT indicate that this technique can provide quantitative data on PBMC aggregates in 3D that can be cross-correlated with human biopsies.
Conclusion: A combination of traditional and emerging imaging modalities can provide new insight into the host-pathogen interaction in tuberculosis.

BS.D.01.06

Single cell analysis reveals functional heterogeneity within plasmacytoid dendritic cells and identifies environmental cues that drive type I IFN production

F. Wimmers[1], *N. Subedi*[2,1,3], *D. Heister*[1], *N. van Buuringen*[1], *J. Vivié*[4], *I. Beeren-Reinieren*[1], *R. Woestenk*[5], *H. Dolstra*[6], *A. Piruska*[7], *A. van Oudenaarden*[4], *C. Figdor*[1], *W. Huck*[7], *J. de Vries*[1], ***T. Jurjen***[2,3,1];
[1]*Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboud university medical center, Nijmegen, Netherlands,* [2]*Lab of Immunoengineering - Dept. Biomedical Engineering - Einhoven University of Technology, Eindhoven, Netherlands,* [3]*Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, Netherlands,* [4]*Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences (KNAW) and University Medical Center Utrecht, Utrecht, Netherlands,* [5]*Laboratory of Hematology - Department of Laboratory Medicine - Radboud Institute for Molecular Life Sciences, Radboud university medical center, Nijmegen, Netherlands,* [6]*Laboratory of Hematology - Department of Laboratory Medicine - Radboud Institute for Molecular Life Sciences, Radboud university medical center, Nijmegen, Netherlands, Nijmegen, Netherlands,* [7]*Department of Physical Organic Chemistry, Institute for Molecules and Materials, Radboud University, Nijmegen, Netherlands.*

Introduction: Cellular heterogeneity emerges as a key feature of type I IFN-mediated antiviral immunity. Little is known about the factors involved in modulating cellular heterogeneity and the influence of the microenvironment. We investigate how cellular heterogeneity and the microenvironment orchestrate the type I IFN response in plasmacytoid dendritic cells (pDCs). Materials and Methods: We developed a droplet-based microfluidic platform and investigated type I IFN production in human pDCs at single-cell level. Furthermore, our platform warrants functional analysis of live single cells under omission of a microenvironment and we combined this with single cell RNA-seq. Results: For the first time in an unbiased approach, we show here in primary human immune cells that an additional deeper level of functional heterogeneity arises upon stimulation of dendritic cells. We demonstrate that type I IFN production by primary human pDCs is stochastically regulated when stimulated individually with pathogen analogues. Less than 1% of pDCs produced type I IFN despite secretion and expression of other activation markers. Importantly, we determined a crucial role for the microenvironment as a paracrine feedforward loop is able to amplify the type I IFN production leading to the well-described robust type I IFN response that pDCs are so famous for.Conclusions: Our unique technology platform revealed that the inflammatory state of the microenvironment and not the stimulus concentration perceived by pDCs determines the response strength. This has important implications for pDC-focused treatment approaches as the main focus of regulation should be in modifying the type I IFN-based paracrine regulatory system.

WORKSHOPS

WS.A1.01 Myeloid lineage specifications

WS.A1.01.01
Tissue resident mast cells are of dual hematopoietic origin

R. Gentek;
Centre d'Immunologie de Marseille-Luminy (CIML), Marseille, France.

Hematopoiesis occurs in spatio-temporally distinct waves. The aorta-gonado-mesonephros (AGM) gives rise to 'definitive' hematopoietic stem cells (HSC) with the potential for all blood lineages. At earlier stages, hematopoietic progenitors emerging in the yolk sac (YS) generate erythrocytes and megakaryocytes, while YS derived erythro-myeloid progenitors give rise to macrophages (Mac). Whether HSC independent YS progenitors also significantly contribute to other lineages is currently unknown. This is at least partially due to the limitations of current lineage tracing models. Here, we established a novel model that enables fate mapping of both HSC independent YS and definitive hematopoiesis in an efficient and precise, temporally defined fashion (Cdh5-CreERT2 fate mapping). Using this tool, we revealed that embryonic mast cells (MC) initially derive from YS precursors, but get progressively replaced by definitive MC. Replacement of YS derived MC occurs with tissue specific kinetics. YS derived embryonic and definitive adult MC differ substantially phenotypically and transcriptomically. Moreover, adult MC are largely independent from the BM at steady state and during replenishment following depletion. These findings challenge the current dogma that MC originate from the BM. Instead, their developmental kinetics are highly reminiscent of Mac. Our work adds MC to the list of lineages with dual origin. Key questions that have remained unanswered or controversial for other lineages thus also apply to MC, such as the relative influence of ontogeny and (micro)environment and the establishment of immune cell niches. Cdh5-CreERT2 fate mapping represents a powerful tool to address these questions and further dissect hematopoiesis.

WS.A1.01.02
Determining dendritic cell ontogeny across organs by cellular barcoding

***T. Tak**, A. Magniez, L. Perié;*
Institut Curie, Paris, France.

Dendritic cells (DCs) are rare cells that are widely distributed throughout the body. A multitude of functions, origins and cellular markers have been attributed to DCs, resulting in a complicated classification. We aimed to determine the ontogeny of the different DC subsets across different organs in the mouse using cellular barcoding, a technique that simultaneously traces the differentiation of many cells *in vivo*. Murine hematopoietic stem and progenitor cells (HSCs and MPPs) were infected with a lentivirus containing DNA barcodes of 100 random nucleotides. This resulted in uniquely barcoded progenitors whose progeny inherit the barcode. HSCs/MPPs were transferred into sub-lethally irradiated C57BL/6 mice. After 2-6 weeks, DC subsets (pDC, cDC1 and cDC2), B cells and neutrophils were isolated from, bone marrow, spleen liver and lungs. Barcodes were identified by PCR and deep sequencing. Since daughter cells from the same progenitor share the same barcode, cell ontogeny could be analysed using hierarchical clustering. After HSC transplantation, detected barcodes were shared across a wide range of cell types, indicating that a single HSC can produce a wide variety of cells. Samples obtained after transplantation of MPPs showed a large proportion of barcodes being shared between different DC subsets within each organ and between DC subsets in liver and lungs. Only few barcodes were shared between DC subsets from spleen and liver/lungs, however, suggesting a different developmental origin of DC subsets in lungs and liver compared to the spleen.

WS.A1.01.03
Bone marrow-resident dendritic cells play an important role in anti-fungal immunity by boosting granulopoiesis upon recognition of yeast particles

*M. Goedhart, E. Slot, M. F. Pascutti, S. Geerman, T. Rademakers, B. Nota, C. Voermans, **M. A. Nolte**;*
Sanquin, Amsterdam, Netherlands.

Systemic infections with yeast and fungi are major causes of morbidity and mortality following hematopoietic stem cell transplantations and in patients with bone marrow (BM) failure. As BM is enriched for memory T cells specific for fungal antigens, we hypothesize that BM is important for anti-fungal immunity.
Here, we focused on dendritic cells (DCs) in murine BM, which largely belong to the IRF4-regulated subtype that is associated with Th2/Th17 immunity. We found that these cells are localized around sinusoids and rapidly activated upon intravenous endotoxin injection. Gene-expression profiling revealed that BM-resident DCs are, compared to the spleen, highly enriched for several c-type lectins, including Dectin-1, which can bind beta-glucans expressed on fungi and yeast. Indeed, DCs in BM were much more efficient in phagocytosis of both yeast-derived zymosan-particles and conidia of Aspergillus compared to their splenic counterparts, which was highly dependent on Dectin-1. DCs in human BM were also able to efficiently take up zymosan, which depended on β1-integrins. Given their localization inside the body's hematopoietic organ, we examined whether BM-resident DCs can also regulate hematopoiesis. Strikingly, we found that zymosan-stimulated BM-resident DCs enhanced the differentiation of hematopoietic progenitor cells towards neutrophils, while also boosting the maintenance of functional progenitors.
Our findings demonstrate that BM-resident DCs play an important role in anti-fungal immunity. The ability of BM-resident DCs to boost granulopoiesis is highly relevant from a clinical perspective, and contributes to our understanding of the susceptibility for fungal infections under conditions of BM damage.

WS.A1.01.04
Common monocyte progenitors are novel antimycobacterial effector cells

***P. Henneke**, A. Lösslein;*
CCI, Freiburg, Germany.

Mycobacterial tissue infections are characterized by the formation of a multicellular granuloma containing specialized immune cells. Granulomas compromise a unique macrophage species, so called multinucleated giant cells (MGC). In this work we dissected the origin of MGC, which has remained largely exclusive, so far. Accordingly, we isolated different monocyte precursor subsets from murine bone marrow (BM) and found the common monocyte progenitor (cMoP) to have the highest potential to form MGC in response to mycobacterial glycolipids or whole bacteria. Next to the established high proliferative activity, cMoP showed striking effector cell characteristics, e.g. robust formation of TNFα and nitric oxide in response to mycobacterial glycolipids. Furthermore, cMoP showed a distinct differentiation pattern *in vitro*. They rapidly downregulated CD117 expression and upregulated CD11b and F4/80 expression, which is in line with the differentiation into macrophages. However, in contrast to the immunophenotype of differentiated macrophages they maintained high potential to form MGC. Transcriptome analysis revealed an increase in cholesterol and fatty acid metabolism in cMoP, representing a metabolic profile, which allows for the formation of lipid bodies and nucleation of mycobacteria in the cytosol. Fatty acid synthase inhibition impaired MGC formation by cMoP, indicating that the metabolic changes are a prerequisite for the transformation program. We hypothesize that cMoP serve as MGC progenitors in local mycobacterial infections. Together, we herewith provide firm evidence that cMoP, which have been hitherto defined as precursors committed to renew monocytes only, act as specific effector cells in antimycobacterial immunity.

WS.A1.01.05
Identification of the unique functional phenotype of iMATE-defining monocytes that drive hepatic T cell proliferation

***K. Pawelka**[1], P. Knolle[1], M. Heikenwälder[2];*
[1]Institute of Molecular Immunology, Munich, Germany, [2]DKFZ, Heidelberg, Germany.

Intrahepatic myeloid-cell aggregates form in response to Toll-like-receptor 9 (TLR9) signaling in a TNF-dependent fashion to provide a unique anatomic structure that drives local proliferation of cytotoxic CD8 T cells (iMATEs) and confers protection against viral infection. Yet, the identity of the iMATE-defining myeloid cell population remained elusive.
We systematically analyzed the phenotype of myeloid cells in the murine liver after TLR9 activation using a set of different methodologies. Initial flow cytometric phenotypic characterization and tSNE analysis revealed a complex composition of monocytes and newly differentiating macrophages that hinted towards a sequential replacement of liver-resident macrophages followed by repopulation through bone marrow derived inflammatory monocytes. Laser-capture microdissection and genome wide analysis of gene expression identified a set of marker proteins that were validated by flow cytometry and led to the definition of a particular phenotype of monocyte-derived macrophages exclusively found in iMATEs but not elsewhere.
Functional assays of these iMATE-defining monocyte-derived macrophages revealed a high potency in the induction of CD8 T cell proliferation, in the differentiation towards GzmB expression rendering them efficient killer cells and in cross-presenting soluble antigens to CD8 T cells. Liver macrophages, in contrast, failed to provide any support for T cell proliferation and did not show significant cross-presentation capacity.
The transient presence of iMATE-defining monocyte-derived macrophages in the liver indicates that protective hepatic T cell immunity is determined by the dynamics of the changes in inhibitory vs stimulatory macrophage populations, which do not fall into the conventional M1/M2 categories but are related to iMATE formation.

WS.A1.01.06
C/EBPα is crucial in early dendritic cell development

A. Muralikrishnan[1], *A. Rosenberger*[1], *E. Schwarzenberger*[2], *H. Strobl*[2], *A. Zebisch*[1], *H. Sill*[1], *A. Wölfler*[1];
[1]*Division of Hematology, Graz, Austria,* [2]*Institute for Pathophysiology and Immunology, Graz, Austria.*

Background: Dendritic cells (DC) are crucial effector cells of the immune system, which are derived from hematopoietic stem cells via distinct progenitors. Briefly, in presence of FLT3L, myeloid progenitors differentiate to monocyte/DC progenitors (MDP) and subsequently to common DC precursors (CDP), which differentiate into all subsets of mature DC. C/EBPα is a crucial transcription factor in myelopoiesis, however, its role in DC development is not well defined. **Methods/Results:** FLT3L-induced DC formation was studied *in vitro* using bone marrow progenitors (BMP) from inducible BM-specific knockout (ko) mice for C/EBPα. In contrast to wildtype (wt) cells, BMP from ko mice exhibited a profound reduction in numbers of formed DC. Specifically, in ko BMP we observed a reduction in the formation of MDP and a block in their transition to CDP, whereas wt BMP nicely underwent transition towards mature DC. Gene expression analysis revealed a significant change in transcription factors associated with DC development like IRF8, inflammatory cytokines like TNFα and IL1β and several genes related to the NFκB pathway. Interestingly, addition of TNFα and, even better, a combination of TNFα and IL1β to BMP cultured *in vitro* with FLT3L restored formation of functionally mature DC in ko cells. **Conclusions:** C/EBPα plays a critical role in early DC development enabling the formation of MDP and their transition to CDP. Activation of the NFκB pathway as well as induction of IRF8 are likely involved in this C/EBPα-dependent mechanism, since inflammatory cytokines, like TNFα, can overcome this early developmental block observed in BMP lacking *Cebpa*.

WS.A2.01 T cells in aging

WS.A2.01.01
Characherisation of IL 10 producing CD4$^+$ T cells in aged mice

I. M. Ogunsulire[1], *M. Almanan*[2], *D. Hildeman*[2], *C. Hölscher*[1];
[1]*Division of Infection Immunology, Research Centre Borstel, Germany, Sülfeld, Germany,* [2]*Division of Immunobiology, Cincinnati Children's Hospital Medical Centre, USA, Cincinnati, United States.*

One of Mankind's greatest triumphs is prolonged life-expectancy. Conversely, this increase in lifespan is accompanied with its own set of problems. One such problem is a weakened or dysregulated immune state that the leads to an increased susceptibility to infections and diseases in the elderly. A major component of immune dysregulation is chronic inflammation, referred to as "inflammaging", and is characterised by elevated systemic level of interleukin (IL)-6. However, mechanisms controlling inflammaging and its effects on the immune system remain unclear. Recent data show that IL6 promotes the accumulation of FoxP3$^-$ CD4$^+$ T cell which produce a major antiinflammatory cytokine, IL-10. So far, the origin and the type of IL-10-producing cells are yet to be determined. Based on cells described in the literature, we envisioned four potential origins of these IL-10-producing cells, namely exTh17, exTregs, type 1 regulatory (Tr1) cells and T-follicular helper (Tfh) cells. Using IL-17 fate mapping reporter mice, we found that roughly 2% of the IL-10-producing cells were exTh17 cells. Again, using FoxP3 fate mapping reporter mice, roughly 25 % of the IL-10 producing cells were exFoxP3$^+$ cells, although this frequency was similar in young and old mice. Next, we characterised the CD4$^+$ FoxP3$^-$ IL-10$^+$ population and found that some expressed canonical markers of Tr1 cells while others expressed TfH markers, with both of these T cell populations producing the most IL10 with age (over 30% in old mice). Future experiments will determine mechanisms by which IL6 promotes accrual of these cells and their impact on immuneaging.

WS.A2.01.02
Peripheral Antibody titers are affected by highly differentiated, senescent, and exhausted T cells, and pro-inflammatory changes in the human Bone Marrow

E. C. Naismith[1], *L. Pangrazzi*[1], *C. Miggitsch*[1], *M. Grasse*[1], *B. Weinberger*[1], *K. Trieb*[2], *B. Grubeck-Loebenstein*[1];
[1]*Institute for Biomedical Aging research, Innsbruck, Austria,* [2]*Klinikum Wels, Wels, Austria.*

Many antigen-experienced immune cells migrate back to the bone marrow (BM), where they can remain in BM niches for an extended period. In this study, a detailed phenotypical and functional characterization of immune cells isolated from human BM was analyzed to determine if the accumulation of highly-differentiated cells limit the accumulation of other immune cells. CD8$^+$ T cells which no longer express the CD28 co-stimulatory molecule and have acquired the expression of replicative senescence markers CD57 and/or KLRG-1, increase in the BM with age. Exhausted PD-1$^+$ T cells are also seen to increase in the BM with age. Senescent cells secrete pro-inflammatory cytokines inducing low-grade chronic inflammation. Using BM and peripheral blood samples from patients undergoing hip replacement surgery, we show that highly differentiated CD8$^+$ T cells in the BM negatively correlated with B cells, and similar correlations were seen for highly-differentiated, pro-inflammatory cytokine-producing CD8$^+$ T cells. In addition, mRNA expression of IL-15 and IFNγ negatively correlated with B cells in the BM. Peripheral Diphtheria antibody titers negatively correlated with highly-differentiated CD8$^+$CD57$^+$ T cells, and Exhausted Central Memory CD8$^+$ and CD4$^+$ T cells in the BM. Senescent p21 and KLRG-1 expressing CD8$^+$ T cells in the BM and PB, as well as ROS production in the BM, also negatively correlated with the Diphtheria Ab titer. In summary, the accumulation and maintenance of highly-differentiated, senescent, and exhausted cells in the BM may negatively effect the maintenance of other immune cell types, and therefore reduce overall immune function.

WS.A2.01.03
Strong homeostatic TCR signals induce formation of self-tolerant virtual memory CD8 T cells

A. Moudra[1], *A. Drobek*[1], *D. Mueller*[2], *M. Huranova*[1], *V. Horkova*[1], *M. Pribikova*[1], *R. Ivanek*[2,3], *S. Oberle*[4,5], *D. Zehn*[4,6], *K. D. McCoy*[7,8], *P. Draber*[1], *O. Stepanek*[1];
[1]*Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic,* [2]*Department of Biomedicine, University Hospital and University of Basel, Basel, Switzerland,* [3]*Swiss Institute of Bioinformatics, Basel, Switzerland,* [4]*Swiss Vaccine Research Institute, Epalinges, Switzerland,* [5]*Sanofi Genzyme, Baar, Switzerland,* [6]*Division of Animal Physiology and Immunology, School of Life Sciences Weihenstephan, Technical University of Munich, Freising, Germany,* [7]*Department of Clinical Research (DKF), University of Bern, Bern, Switzerland,* [8]*Department of Physiology and Pharmacology, Cumming School of Medicine, University of Calgary, Calgary, Canada.*

Virtual memory T cells are foreign antigen-inexperienced T cells that have acquired memory-like phenotype and constitute 10-20% of all peripheral CD8$^+$ T cells in mice. Their origin, biological roles, and relationship to naïve and foreign antigen-experienced memory T cells are incompletely understood. By analyzing TCR repertoires and using retrogenic monoclonal T-cell populations, we demonstrate that the virtual memory T-cell formation is a so far unappreciated cell fate decision checkpoint. We describe two molecular mechanisms driving the formation of virtual memory T cells.
First, virtual memory T cells originate exclusively from strongly self-reactive T cells. Second, the stoichiometry of the CD8 interaction with Lck regulates the size of the virtual memory T-cell compartment via modulating the self-reactivity of individual T-cells. Although virtual memory T cells descend from the highly self-reactive clones and acquire a partial memory program, they are not more potent in inducing experimental autoimmune diabetes than naïve T cells. These data underline the importance of the variable level of self-reactivity in polyclonal T cells for the generation of functional T-cell diversity.

WS.A2.01.04
Extreme memory inflation of HLACw0702 restricted CMV specific CD8+ T cells that dominate the CD8+ T cell repertoire during healthy ageing

L. Hosie[1], *A. Pachnio*[1], *J. Zuo*[1], *H. Pearce*[1], *S. Riddell*[2], *P. Moss*[1];
[1]*Institute of Immunology and Immunotherapy, Birmingham, United Kingdom,* [2]*Fred Hutchinson Cancer Research Center, Seattle, United States.*

Introduction Cytomegalovirus (CMV) elicits a strong T-cell immune response which increases during aging in a process termed 'memory-inflation'. CMV downregulates HLA-A/-B molecules on the surface of CMV-infected cells to limit presentation of viral peptides to T-cells. Comparatively, HLA-C is relatively spared and engages with inhibitory KIR receptors to reduce lysis by NK cells. **Methods** The magnitude and functional properties of CMV-specific CD8+T-cells specific for HLA-C-restricted peptides were investigated in a cohort of 53 donors aged 23-91 years. This was achieved via peptide stimulation of PBMCs followed by multi-colour flow cytometry. **Results** Three HLA-Cw*0702-restricted peptides, derived from immediate-early expressed proteins, elicited strong immune responses which increased substantially with age such that the average aggregate response represented 37% of the CD8+T-cell pool within donors >70 years of age. This aggregate reached 70.3% TNF-α$^+$ and remarkably, a single IFN-γ$^+$ response represented 70% of the total CD8+T-cells of a 91-year old donor at the most extreme. HLA-Cw*0702-restricted CD8+T-cell responses did not show features of exhaustion such as CD39 expression. Indeed they exhibited a polyfunctional Th1 cytokine profile and cytotoxic phenotype expressing high levels of dual perforin$^+$granzymeB$^+$ that became exaggerated with age. Functionally, HLA-Cw*0702-restricted PBMCs showed exceptionally high avidity for cognate peptide-HLA demonstrating early and efficient recognition of CMV-infected cells. **Conclusions** These observations indicate that HLA-C-restricted CD8+T-cells C play an important role in the control of latent CMV infection representing a novel opportunity for CD8+T-cell therapy of CMV reactivation within immunosuppressed patients. The efficacy of such a therapy is currently being investigated via TCR transgenics.

WS.A2.01.05

Age-Related Decline in Primary CD8$^+$ T Cell Responses is Associated with the Development of Senescence in a Subset of Antigenically Naive CD8$^+$ T Cells

N. L. La Gruta[1], *K. M. Quinn*[1], *A. Fox*[2], *K. L. Harland*[2], *B. E. Russ*[3], *J. Li*[3], *T. H. Nguyen*[2], *L. Loh*[2], *M. Olshansky*[3], *H. Naeem*[4], *K. Tsyganov*[4], *F. Wiede*[1], *R. Webster*[1], *C. Blyth*[1], *X. Y. Sng*[1], *T. Tiganis*[1], *D. Powell*[4], *P. C. Doherty*[2], *S. J. Turner*[3], *K. Kedzierska*[2];
[1]*Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Clayton, Australia,* [2]*The Doherty Institute for Infection and Immunity and Department of Microbiology and Immunology, University of Melbourne, Melbourne, Australia,* [3]*Monash Biomedicine Discovery Institute and Department of Microbiology, Monash University, Clayton, Australia,* [4]*Bioinformatics Platform, Monash University, Clayton, Australia.*

Ageing undermines primary CD8 T cell responses and this occurs, in part, due to direct effects on naïve CD8 T cells to reduce intrinsic functionality, but the precise nature of any intrinsic defect and its molecular basis remains to be defined. Ageing also causes accumulation of antigen-naïve but semi-differentiated "virtual memory" (T_{VM}) cells, recently identified in humans, but their contribution to age-related functional decline is unclear. Here, we show that T_{VM} cells become nearly completely non-proliferative in aged mice and humans, despite being highly proliferative in young individuals. In contrast, conventional naïve T cells (T_N cells) retain almost complete proliferative capacity in both aged mice and humans. Adoptive transfer experiments in mice illustrated that the proliferative dysfunction acquired by T_{VM} cells was imposed by the aged environment and could not be rescued by maintenance in a young environment. Despite previous studies to the contrary, transcriptional analyses did not implicate exhaustion in aged T_{VM} cell dysfunction. Rather, these cells exhibited a profile consistent with senescence, with increased Bcl-2 expression and phosphorylation of γ-H2AX in steady state, along with increased *Cdkn1a* (p21) expression and defective cyclin D1 accumulation after TCR stimulation. Collectively, this study marks the first description of senescence in an antigenically naïve T cell population, and highlights markedly different impacts of ageing on distinct T cell populations. Consequently, this work has implications for the targeting of distinct T cell populations in current immunotherapies.

WS.A2.01.06

Influence of aging on calcium signals and cytotoxicity in murine CD8$^+$ T cells

A. Angenendt[1], *R. Steiner*[1], *A. Knörck*[1], *G. Schwär*[1], *E. Krause*[2], *A. Lis*[1];
[1]*Biophysics, CIPMM, Saarland University, Homburg, Germany,* [2]*Physiology, CIPMM, Saarland University, Homburg, Germany.*

Cytotoxic T lymphocytes (CTLs) are key players in the adaptive immune response and several steps of the CTL killing machinery require or are modulated by Ca^{2+} itself. The major route of Ca^{2+} influx in lymphocytes is through store-operated calcium entry (SOCE). CTL function is altered *in vivo* and *in vitro* in elderly compared to adult individuals and the immune response is compromised with progressing age. To investigate, whether reduced expressions of SOCE components, stromal interaction molecule (STIM) and Orai, contribute to Ca^{2+} signal reductions in CD8$^+$ T cells from elderly mice, we performed flow cytometry, electrophysiology and molecular biology experiments with murine CD8$^+$ T cells from an adult and an elderly age group. Furthermore, we compared their killing kinetic and efficiency using a time-resolved killing assay, investigated the Ca^{2+}-dependency of the process, and quantified the expression of relevant proteins involved in cytotoxicity. We were able to link the reduced Ca^{2+} signals and Ca^{2+} release-activated Ca^{2+} currents of CD8$^+$ T cells from elderly mice to a decrease in mRNA and protein levels of STIMs and Orais. Moreover, the reduced Ca^{2+} signals of stimulated CD8$^+$ T cells from elderly mice are not due to differences in subtype distribution between both age groups, but rather in the most abundant CD8$^+$ T cell subtypes, central and effector memory. Strikingly, we found that CD8$^+$ T cells from elderly mice show an altered expression pattern of relevant proteins involved in killing machinery correlating with improved cytotoxicity but minor Ca^{2+}-dependency.

WS.A2.02 Immune development and neonatal responses

WS.A2.02.01

Dynamic transcriptome-proteome correlation networks contribute to human myeloid differentiation and neutrophil development

A. J. Hoogendijk[1], *M. van den Biggelaar*[1], *F. Pourfarzad*[2], *C. Aarts*[2], *A. Tool*[2], *I. Hiemstra*[2], *F. van Alphen*[1], *C. van der Zwaan*[1], *T. K. van den Berg*[2], *L. Grassi*[3], *M. Frontini*[3], *W. H. Ouwehand*[3], *A. B. Meijer*[1], *T. W. Kuijpers*[2];
[1]*Department of Plasma Proteins, Sanquin Research, Amsterdam, Netherlands,* [2]*Department of Blood Cell Research, Sanquin Research, Amsterdam, Netherlands,* [3]*Department of Haematology, University of Cambridge, Cambridge, United Kingdom.*

Neutrophils are the most abundant leukocytes in human blood and form the frontline of the innate host-response to bacterial and fungal infections. To fulfill this role, they exert a wide array of pathogen eliminating actions. During neutrophil development, transcriptional programs and protein production are induced to enable these actions.
Using mass spectrometry-based quantitative proteomics combined with previously obtained transcriptomics data, we unraveled the dynamic transcriptome and proteome changes that accompany the metamorphosis from four developmental stages in the bone marrow into a mature distinct non-dividing polymorphonuclear blood cell.
We identified 2429 transcript-protein pairs that were differentially expressed on either RNA, protein or on both levels, which were used to assemble a co-expression network comprising of 12 modules. These included transcript-protein dynamics following the classical dogma of protein levels lagging behind changes in RNA expression. In contrast, we also observed modules with discordant dynamics, e.g. increased RNA expression at the final stages of differentiation, with no changes in protein level. Importantly, the transcript-protein dynamics of most modules could directly be linked to functional aspects of neutrophil development and mature neutrophil functions; such as: modules containing decreased mitochondrial process, increased immune response signatures; or modules exhibiting high association with specific neutrophil granules.
Therefore, this comparison of proteome with transcriptomic data unveiled highly dynamic and differential interactions between RNA and protein kinetics during human neutrophil development linked to functional aspects of myeloid development and the typical end-stage blood neutrophil features including morphology and killing activities.

WS.A2.02.02

CircRNA expression is a highly regulated process during hematopoiesis and lymphocyte differentiation

B. P. Nicolet, *S. Engels, F. Aglialoro, E. van den Akker, M. von der Lindern, M. C. Wolkers;*
Sanquin Research, Amsterdam, Netherlands.

Lymphocyte differentiation during hematopoiesis is dependent on micro-RNAs and long-non-coding RNAs that - in addition to transcription factors - drive the cellular differentiation. Circular RNA (circRNA), another recently identified type of RNA, was also shown to determine vital cellular functions in many cell types. Whether and how circRNAs regulate immune cells is not yet described. Here we investigated the circRNA expression in the hematopoietic tree. Clustering based on circRNA expression recapitulated the differentiation stages in hematopoiesis. Furthermore, correlating the circular with the linear RNA expression revealed that circRNA usage is differently regulated during hematopoiesis. Interestingly, circRNA expression is significantly higher in lymphocytes than in other hematopoietic cells. Analysis of circRNA expression in naïve and memory CD4+ and CD8+ T cells revealed higher circRNA expression in naïve than in memory T cells. Further, we found that CD4+ and CD8+ maturation is marked by 100's of differentially expressed circRNA. We show here for the first time that circRNAs are differentially expressed during hematopoiesis and T cell differentiation, which points to a novel regulatory layer in lymphocyte differentiation.

WS.A2.02.03

IL-2-vs. IL-15-dependent intrathymic development of regulatory T lymphocytes

C. APERT[1], *N. McJannett*[1,2], *P. Romagnoli*[1], *J. van Meerwijk*[1];
[1]*INSERM UMR1043 Centre de Physiopathologie Toulouse Purpan, TOULOUSE, France,* [2]*Keele University, Staffordshire, United Kingdom.*

Natural regulatory T cells (nTreg) develop in the thymus and are defined by their expression of the master regulator transcription factor Foxp3 as well as by their high self-reactivity, which enables them to prevent autoimmune diseases. Peripheral nTreg are heterogeneous. The distinct Treg-subsets display different degrees of self-reactivity, express different transcription factors and chemokine-receptors, and inhibit peripheral immune-responses using distinct effector mechanisms. Among the various signals controlling Treg development in the thymus, the γ_c-cytokines IL-2 and IL-15 apparently play an important role in the survival of these cells. We investigate the roles of these two cytokines in the development of distinct nTreg subsets. By using IL-2- and IL-15-deficient mice harboring a Rag2-GFP transgene that allows discrimination between developing Tregs from peripheral mature cells that had recirculated to the thymus, we confirmed that these two cytokines play quantitatively important and non-redundant roles in the development of Tregs: Treg development is decreased in absence of one or the other cytokine and virtually abolished in absence of both. Single cell RNAseq and multicolor cytometry analysis revealed that these cytokines also have a qualitative effect on Tregs favoring development of in part distinct Treg-subsets. Currently we are assessing the TCR repertoires and immunosuppressive function of Tregs that develop in absence of either of the two cytokines. A better understanding of Treg-development will be of a great importance for targeting these cells in various diseases where they play a beneficial or deleterious role.

WS.A2.02.04

Recent thymic emigrants in neonates and adults share an antimicrobial molecular signature

M. L. Pekalski[1], *A. Rubio Garcia*[1], *A. Paterou*[1], *R. C. Ferreira*[1], *D. B. Rainbow*[1], *A. J. Cutler*[1], *J. L. Jones*[2], *J. A. Todd*[1], *L. S. Wicker*[1];
[1]*Wellcome Centre for Human Genetics, University of Oxford, Oxford, United Kingdom,* [2]*Cambridge Neuroscience, University of Cambridge, Cambridge, United Kingdom.*

The neonatal immune system has to balance tolerance to commensal antigens and yet be able at this critical time to fight pathogenic infections. Importantly, owing to the large and active thymus at birth as opposed to adults, neonatal naive T compartment consist mostly of recent thymic emigrants (RTEs). However the role that recent thymic emigrants have in the early life period is still relatively unexplored. We studied 20 adult donors to achieve 80% power to detect 2-fold differences in gene expression between highly-purified naïve T cell subsets of different cellular age within each donor i.e. the youngest T cells were RTEs and the oldest were naïve T cells that have spent decades outside the thymus.
A unique gene-expression signature in RTEs was confirmed by FACS protein investigation of 389 donors *ex vivo* and in functional studies including RNA-seq and FACS analysis of activated RTEs from the blood of babies, children and adults. We also studied RTEs arising directly from the thymus post lympho-depletion. We showed that complement receptors CR1 and CR2, which are known to bind complement C3b- and C3d-decorated microbial products, are hallmarks of RTEs. These most naïve T cells also express transcripts encoding the LPS-degrading enzyme, AOAH, and TLR1. Following activation RTEs in babies and adults produce IL-8 (CXCL8), a major chemoattractant for neutrophils in bacterial defense.
In conclusion, although RTEs underpin the adaptive arm of the immune system, these cells have innate, anti-bacterial functions that could help orchestrate the balance between neonatal tolerance and microbial defence.

WS.A2.02.05

Characterising the maturation of T cell polarisation in preterm and term infants and in the neonatal chronic lung disease bronchopulmonary dysplasia

J. C. Lao[1], *M. A. Pang*[1], *A. Malhotra*[2], *K. Tan*[2], *F. Beker*[3], *K. Konig*[3], *C. Collins*[3], *C. Theda*[4], *O. Kamlin*[4], *P. J. Berger*[5], *A. Veldman*[1], *C. A. Nold-Petry*[5], ***M. F. Nold***[1,6,2];
[1]*Monash University, Clayton, Australia,* [2]*Monash Newborn, Clayton, Australia,* [3]*Mercy Hospital, Heidelberg, Australia,* [4]*Royal Women's Hospital, Parkville, Australia,* [5]*Hudson Institute, Clayton, Australia,* [6]*Hudson Institute, Melbourne, Australia.*

Background: Bronchopulmonary dysplasia (BPD) is a common neonatal lung disease that is underpinned by pulmonary inflammation. Despite extensive research, a dominant immune pathway that drives BPD remains elusive.
Methods: Cord and peripheral blood was collected from: i) infants born at 24-29 gestational weeks at birth, on days 1, 7 and 14, and at 36 weeks gestational age; ii) healthy term infants at birth and at 4-16 weeks of age; iii) healthy adults. Following overnight stimulation with PMA+ionomycin or vehicle, the Th1/2/17 and regulatory T cell (Treg) subsets were enumerated by flow cytometry. Results were analysed against BPD status and clinical events such as respiratory support, sepsis and medication.
Results: 51 preterm infants, 20 term infants and 5 adults were enrolled, and 258 unique samples collected. Th2 polarisation predominated in preterm and term infants up to 16 weeks of age, with up to 62% of CD4+ T cells Th2-polarised vs 2% in adults. Baseline Th1 and Th17-polarisation was low in all groups; inducibility of Th1 and Th17-polarisation developed at 16 weeks of age. The percentage of Treg was 5-fold higher in infants than in adults. The 36 preterm infants who suffered from BPD exhibited up to 36-fold increased frequencies of Th2-polarised T cells at most timepoints.
Conclusions: Our study sheds light on the maturation of the immune system in preterm and term infants. The severe chronic lung disease BPD is associated with strong Th2 polarisation, suggesting that therapies targeting the Th2 pathway may offer benefit to the tiny BPD patients.

WS.A2.02.06

CD4$^+$ T cells from human neonates and infants respond extensive against antigens of *Candida albicans* and *Aspergillus fumigatus*

K. Vogel[1], *M. Pierau*[1], *A. Arra*[1], *K. Lampe*[2], *D. Schlueter*[3,4], *C. Arens*[2], *M. C. Brunner-Weinzierl*[1];
[1]*Department of Experimental Pediatrics, Magdeburg, Germany,* [2]*Department of Otorhinolaryngology, Head and Neck Surgery, Magdeburg, Germany,* [3]*Institute of Medical Microbiology and Hygiene, Magdeburg, Germany,* [4]*Organ-specific Immune Regulation, Helmholtz Centre for Infection Research, Braunschweig, Germany.*

Fungi are widespread in nature and often colonize human body surfaces. Rarely, an invasive pathology is initiated, but the figure is on the rise. Besides immune suppressed individuals, especially neonates are most susceptible to fungus pathology. Therefore, a better understanding of antifungal responses especially in neonates and infants is essential. Here, we show that antifungal CD4 T-cell responses are initiated from birth on to monocyte-derived antigen presenting cells pulsed with *Candida albicans* or *Aspergillus fumigatus* lysates and fungal peptide pools, respectively. We found that, the neonatal responding T-cell pool constitute 20 out of 24 different TCR-V_β families whereas infant and adult pools display significantly less TCR-V_β-variability. In comparison to adults, naïve T cells from neonates and infants proliferated at a 4-5 times higher frequency in response to *C. albicans*, which was also corroborated by an immediate co-expression of multiple cytokines. Frequencies of unglycosylated IL-4 producers were similarly high at any age in response to *A. fumigatus*, implicating the activation of Th2-machinery, not biased due to developmental age differences. Frequencies of IL-17 producers were about 3 times higher among neonates compared to adults. Notable, only T cells from neonates and infants could co-express transcription factors T-bet and RORγt and eventually co-express their target genes IL-17 and IFNγ, implicating a high plasticity of T cell responses in early life. These observations clearly confirm the age-dependent characteristics of antifungal T-cell responses. In this way, our findings will contribute to optimizing antifungal therapies that were previously limited to antimycotics with severe side effects.

WS.A2.03 Immune cell aging and differentiation

WS.A2.03.01

Identification of age-related immune signatures by mass cytometry

J. Braun, *S. Schlickeiser, A. Andrzejewska, F. Papatzika, B. Kruse, S. Geissler, A. Arampatzis, A. Thiel;
Berlin-Brandenburg Center for Regenerative Therapies, Berlin, Germany.*

Human biological aging is associated with functional physiological deteriorations and increasing systemic inflammation and many cellular, immunological alterations have been reported. Due to technical limitations, these reports focused mostly on specific cell populations, e.g. only T cells were assessed. However, for a systematic understanding of age-related immune signatures, all different cell subsets should be evaluated and associated with medical and functional physiological parameters. Therefore, we have performed an integrated study combining mass cytometry, multiplex ELISA and biomechanic measurements to characterize cohorts of healthy young, healthy elderly and frail elderly participants. Fresh PBMCs were analyzed by mass cytometry (37 antibodies). We applied an analysis pipeline for Automated Comprehensive Immunophenotying and Subset Enumeration (ACISE). Here, cell populations are identified by over-clustering using SPADE, followed by characterization based on user-defined cutoffs for marker positivity, and annotation according to user-specified target phenotype definitions as well as subset hierarchies (similar to conventional gating analysis). We identified not only already reported fluctuations in CD8 TEMRA, CD4 recent thymic emigrants, or non-classical monocytes, but also changes in regulatory T cells, NK cells and B cells. Some changes were exclusively and significantly observed within frail participants. Our study may represent the first comprehensive mass cytometric analysis of immune signatures including clearly separated cohorts of frail and healthy elderly. Furthermore, the applied ACISE analysis pipeline is a helpful tool for the analysis of multi-dimensional single cell data, as it allows semi-automated categorization of cell subsets into parental immune cell subsets and calculation of subset frequencies accordingly.

WS.A2.03.02

Aging affects the frequency, functions and antibody repertoire of human B-1 cells

A. M. Hernández-Vázquez[1], *N. Rodríguez-Zhurbenko*[1], *T. Quach*[2], *T. J. Jopkings*[2], *T. L. Rothstein*[3];
[1]*Center for Molecular Immunology, Havana, Cuba,* [2]*Feinstein Institute for Medical Research, New York, United States,* [3]*Center for Immunobiology of the Western Michigan University Homer Stryker M.D. School of Medicine, Kalamazoo, United States.*

Introduction: Aging decreases the efficiency of several immune functions, which has been associated with an increased incidence of infections, autoimmune diseases and cancer. Human naïve and memory B cells suffer significant quantitative and qualitative changes in the elderly. However, human B-1 cells, which play critical housekeeping and anti-microbial defensive roles, have not been studied. In the present work we analyzed how the frequency and function of human B-1 cells (CD20+CD27+CD43+CD38low) change with age.
Materials and Methods: The frequency of human B-1 cells was studied by flow cytometry. The capacity of these cells to produce IgM was detected by ELISPOT and their repertoire and genes related with Ig secretion were studied by single cell PCR.
Results: Our results show that the percentage of B-1 cells, but also their capacity to spontaneously secrete IgM decrease with age. Expression levels of Xbp1 and Blimp1, associated with Ab secreting phenotype, were significantly lower in elderly donors, while Pax5, characteristic of non-secreting B cells, was significantly higher. Furthermore, elderly donors showed a reduction of the antibody repertoire variability in comparison with young individuals. It was interesting to observe differences in the usage of certain VH and DH families among young and elderly individuals. We also proved that B-1 cells are involved in the secretion of antibodies against NeuGcGM3 ganglioside, a tumor associated antigen, and this specificity also decreases with age.
Conclusions: These results show changes in the frequency and function of human B-1 cells with aging, which could affect their protective and homeostatic functions.

WS.A2.03.03

T cell immunity does not age in a long-lived rodent species

M. Izraelson[1], *T. Nakonechnaya*[1,2,3], *A. Davydov*[4], *M. Dronina*[5], *D. Miskevich*[5], *I. Mamedov*[1], *L. Barbashova*[2], *M. Shugay*[1,3,6], *D. Bolotin*[1], *D. Staroverov*[1], *E. Kondratyuk*[7], *S. Lukyanov*[3], *I. Shams*[5], *O. Britanova*[1], *D. Chudakov*[1,6,4];
[1]*Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russian Federation,* [2]*Nizhny Novgorod State Medical Academy, Nizhny Novgorod, Russian Federation,* [3]*Pirogov Russian National Research Medical University, Moscow, Russian Federation,* [4]*Central European Institute of Technology, Brno, Czech Republic,* [5]*Institute of Evolution & Department of Evolutionary and Environmental Biology at University of Haifa, Haifa, Israel,* [6]*Center for Data-Intensive Biomedicine and Biotechnology, Skolkovo Institute of Science and Technology, Moscow, Russian Federation,* [7]*Institute of Systematics and Ecology of Animals SB RAS, Novosibirsk, Russian Federation.*

The T cell receptor (TCR) diversity of naïve T lymphocytes represents a precious collection of keys from which antigen-specific variants are selected, conferring protection for the host against new challenges. Here we analyzed peripheral TCR repertoires from humans, mice, and blind mole-rats (*Spalax spp.*)—long-lived, cancer-resistant rodents. We report that *Spalax* T cell diversity remains stable even for animals that reach extreme old age (15-17 years), in striking contrast to mice and humans, for whom immunosenescence is associated with the accumulation of large numbers of memory clones. This discovery reveals a distinctive strategy for T cell immunity organization that potentially underlies the extraordinary longevity of *Spalax*, and encourages a careful re-examination of the contribution of immunosenescence to life span in mammals.The work was supported by the Russian Science Foundation project № 16-15-00149 (to BOV). Mice samples preparation was supported by grant of the Ministry of Education and Science of the Russian Federation.

WS.A2.03.04

Antigen-specific immunity is inhibited in the skin of older people by p38 MAPK-driven inflammatory monocytes

E. S. Chambers[1], *M. Vukmanovic-Stejic*[1], *H. Trahair*[1], *A. Appios*[1], *V. Birault*[2], *M. Noursadeghi*[1], *N. Mabbott*[3], *M. Rustin*[4], *A. Akbar*[1];
[1]*University College London, London, United Kingdom,* [2]*The Francis Crick Institute, London, United Kingdom,* [3]*The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh,, Edinburgh, United Kingdom,* [4]*Royal Free Hospital, London, United Kingdom.*

Decline in immunity with age is often associated with an increase in low grade chronic inflammation, termed 'inflammageing'. We have shown that there is a large proinflammatory response to a saline injection in the skin of older (>65 years) people which is not observed in the young (<40 years). Bioinformatic and immunohistochemical analysis demonstrated that the inflammatory saline response is driven by recruitment of inflammatory monocytes (CD14+CCR2+) and a p38-MAPK inflammatory gene signature. Importantly this inflammatory response to saline is negatively correlated with an antigen specific response in the skin to VZV.
In this study we investigated if pre-treatment with a small molecular oral inhibitor for p38-MAPK, Losmapimod, would improve response to VZV challenge and decrease the inflammatory response to saline in older individuals. Losmapimod reduced systemic inflammation (CRP serum levels) and significantly enhanced cutaneous responses to VZV antigen challenge in the same individuals ($p < 0.0006$). The increase in VZV clinical score post-Losmapimod treatment correlated with an increase in the number of T cells (CD4+ and CD8+) in the skin. In those individuals with increased T cell infiltrate, there was reduced inflammatory monocyte infiltration at 6 hours.
To conclude this data proposes that inflammatory monocytes significantly inhibit antigen-specific immunity in the skin of older people. Additionally, pre-treatment with the systemic p38-MAPK inhibitor Losmapimod inhibits inflammatory monocyte infiltration and restores antigen-specific immunity.

WS.A2.03.05

Screen of epigenetic inhibitors reveals two novel T helper 17 (Th17) cell regulators: BRPF1 and BRPF2

L. Chen[1], *N. Zaarour*[1], *T. Sekine*[1], *M. Sundstrom*[2], *M. Feldmann*[3], *A. Cribbs*[1], *F. McCann*[4], *P. Bowness*[1];
[1]*Botnar Research Centre, Oxford, United Kingdom,* [2]*SGC Karolinska, Solna, Sweden,* [3]*The Kennedy Institute of Rheumatology, University of Oxford, United Kingdom,* [4]*The Kennedy Institute of Rheumatology, Oxford, United Kingdom.*

Introduction: T helper 17 (Th17) lymphocytes, defined by the production of interleukin (IL)-17A, have been implicated in the pathogenesis of several common inflammatory arthritides including rheumatoid arthritis, psoriatic arthritis (PsA) and ankylosing spondylitis (AS), with anti-IL-17A antibodies effective in treating patients with PsA and AS. Oral inhibitors for such responses are however still lacking. Through the unbiased screen of epigenetic inhibitors using CD4+ T cells from AS patients, we aim to reveal novel mechanisms of epigenetic regulation in Th17 cells and identify potential therapeutic targets for Th17 pathogenicity.
Methods: A Th17 differentiation/expansion assay using CD4+ T cells from patients with AS was used to screen a library of epigenetic inhibitors. Th17% and IL-17A cytokine production were measured using flow cytometry and ELISA respectively. siRNA gene knockdown was used for target validation, RNA-seq and qPCR were used to investigate downstream pathways.
Results: The Th17 assay screen identified a novel Th17 inhibitor, OF-1. Among the targets of OF-1, BRPF1 and BRPF2, but not BRPF3, were required for Th17 responses. Knockdown of BRPF1 indirectly suppressed Th17 response through promoting the expression of Th17 negative regulators: EGR2, DUSP2 and IL-2. In contrast, BRPF2 silencing directly reduced the expression of Th17-associated genes, including RORC and IL23R.
Conclusions: We have identified OF-1, a BRPF1-3 inhibitor, as a potent and selective epigenetic suppressor for Th17 cells. BRPF1 and BRPF2 regulate Th17 response through distinct mechanisms.

WS.A2.03.06

Differentiation of human Langerhans cells from monocytes and their specific function in inducing IL-22-specific T helper cells

Y. Otsuka[1,2], *E. Watanabe*[1], *E. Shinya*[1], *H. Saeki*[2], *T. B. Geijtenbeek*[3], *H. Takahashi*[1];
[1]*Department of Microbiology and Immunology, Nippon Medical School, Tokyo, Japan,* [2]*Department of Dermatology, Nippon Medical School, Tokyo, Japan,* [3]*Department of Experimental Immunology, Amsterdam Infection & Immunity Institute, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands.*

Human mucosal tissues and skin contain two distinct types of dendritic cell (DC) subsets, epidermal Langerhans cells (LCs) and dermal DCs, which can be distinguished by the expression of C-type lectin receptors Langerin and DC-SIGN, respectively. Although peripheral blood monocytes differentiate into these distinct subsets, monocyte-derived LCs induced by co-cultured with GM-CSF, IL-4 and TGF-β1 (moLCs) co-express both Langerin and DC-SIGN, suggesting that the environmental cues remain unclear. Here we show that LC differentiation is TGF-β1-dependent and that co-factors such as IL-4 and TNF-α promote TGF-β1-dependent LC differentiation into Langerin$^+$DC-SIGN$^-$ moLCs but continuous exposure to IL-4 blocks differentiation. Steroids such as Dexamethasone (Dex) greatly enhanced TNF-α-induced moLC differentiation and blocked DC-SIGN expression. Strikingly, CD1a triggering with squalene on moLCs but not moDCs induced strong IL-22-producing CD4$^+$ helper T cell responses. As IL-22 is an important cytokine in the maintenance of skin homeostasis, these data suggest that CD1a on moLCs is involved in maintaining the immune barrier in skin.

WS.A2.04 Evolution of immune responses in health and disease

WS.A2.04.01

Hematopoietic progenitors from old humans are metabolically active but present evidence of cellular senescence and pyroptosis

T. Fali[1], *V. Fabre Mersseman*[1], *T. Yamamoto*[2], *J. Boddaert*[1,3], *D. Sauce*[1], ***V. Appay***[1,4];
[1]*INSERM CIMI, Paris, France,* [2]*National Institutes of Biomedical Innovation, Osaka, Japan,* [3]*AP-HP, Service de Gériatrie, Paris, France,* [4]*Kumamoto University, IRCMS, Kumamoto, Japan.*

The maintenance of effective immunity over time is dependent on the capacity of hematopoietic stem cells (HSC) to sustain the pool of immunocompetent mature cells. Decline of immune competence with old age may stem from HSC defects, including reduced self-renewal potential and impaired lymphopoiesis, as suggested in murine models. To get further insights into aging related alteration of hematopoiesis, we performed a comprehensive study, including phenotypic, transcriptomic and functional assessments, of blood hematopoietic progenitor cells (HPC) from elderly humans (i.e. >75 years old). In the elderly, HPC present active oxidative phosphorylation and are pressed to enter cell cycling. However, p53-p21 and p15 cell senescence pathways, associated with telomerase activity deficiency, strong telomere attrition and oxidative stress, are engaged, thus limiting cell cycling. Moreover, survival of old HPC is impacted by pyroptosis, an inflammatory form of programmed cell death. Last, telomerase activity deficiency and telomere length attrition of old HPC may be passed on progeny cells such as naïve T lymphocytes, highlighting further the poor lymphopoietic potential and capacity to induce T-cell responses of the elderly. This pre-senescent profile is characteristic of the multiple intrinsic and extrinsic factors affecting HPC in old individuals and represents a major obstacle in terms of immune reconstitution and efficacy with advanced age.

WS.A2.04.02

IMMUNOBIOGRAM a new immunological tool to personalize immunosuppressive therapy in kidney transplant recipients

M. Di Scala[1], *J. Portolés*[2], *C. Jiménez*[3], *D. Janeiro*[2], *E. González*[3], *B. Sánchez Sobrino*[2], *M. López Oliva*[3], *J. Richter*[1], *A. Ortega*[1], *J. Pascual*[4], *I. Portero*[1];
[1]*BIOHOPE Scientific SL, Madrid, Spain,* [2]*Hospital Universitario Puerta de Hierro, Nephrology Department, Madrid, Spain,* [3]*Hospital La Paz, Nephrology Department, Madrid, Spain,* [4]*Hospital del Mar, Nephrology Department and Kidney Transplantation, Barcelona, Spain.*

INTRODUCTION: Transplant rejection is one of the biggest limitations in kidney transplantation(KT). It remains essential to control the immune-mediated tissue-specific destruction using immunosuppressive drugs (IMSs) that limit immune system hyperactivation and prevent the allograft loss.
IMSs regimens are stablished based on standard clinical guidelines and empirically.

BIOHOPE is developing a blood-based Precision Medicine test for KT. It offers a personalized comparative evaluation of patient sensitivity to a panel of IMSs most commonly used. This functional pharmacodynamic and monitoring kit is named Immunobiogram (IMBG), an immunoassay based on the concept of the antibiogram.
METHODS: IMBG is a 3D-cell culture of PBMCs included in a hydrogel capable of spontaneous generation of IMSs concentration-gradient due to a passive-diffusion process. An indicator of cell-proliferation/viability reveals the capacity of IMSs-gradient to inhibit the activation cells state.
RESULTS: IMBG was evaluated in BH-pilot study performed in two major Hospitals in Spain. It included 70 patients belonging to three immunological risk-categories (low-risk, standard, high-risk patients).
The resulting profiles were used to ascertain the sensitivity of each patient to each IMSs and to establish a panel of IMSs recommendation for the clinical management. Significantly associated low-sensitivity patterns have been observed in patients who present worse clinical evolution.
CONCLUSIONS: IMBG provides an automatized method to quantitatively measure the response of a patient to IMSs that will aid clinicians in the determination of the optimal combination/posology of IMSs/immune-modulator drugs and opens the possibility to make the necessary adjustments in immunosuppressive therapy to avoid chronic rejection and reduce side-effects of IMSs.

WS.A2.04.03
Particular neutrophils mediate environment-driven onset of airway allergy through NETs release

C. Radermecker[1]*, C. Sabatel*[1]*, S. Johnston*[2]*, M. Toussaint*[1]*, F. Bureau*[1]*, T. Marichal*[1]*;*
[1]*GIGA-R, Cellular and Molecular Immunology, Sart-Tilman, Belgium,* [2]*Imperial College, London, United Kingdom.*

Environmental changes are responsible for the dramatic rise in the prevalence of allergic asthma worldwide. Decreased exposure to microbial products such as lipopolysaccharide (LPS) and respiratory viral infections represent two major risk factors for asthma, yet the mechanisms linking such conditions and host allergic susceptibility remain unclear. First, we developed two mouse models, a virus-induced asthma model and a model of asthma promoted by exposition to low LPS doses. In these models, only previously infected mice or mice exposed to low LPS doses displayed the characteristics of asthma following sensitization and challenge to house dust mite (HDM). Then, using single-cell RNA sequencing, we found that pro-allergic environments (low LPS doses and respiratory virus) induced the recruitment into the lungs of $CXCR4^{hi}CD49d^{high}LAMP\text{-}1^{high}$ neutrophils releasing neutrophil extracellular traps (NETs). The role of NETs in asthma onset was then demonstrated using three NETosis inhibitors in our two models. Infected or low LPS doses exposed mice exhibited strong decrease of all asthma features when treated with NETs inhibitors compared to non-treated mice. Finally, to address how NETs promote the development of a Th2 immune response, we analysed by flow cytometry the distinct subpopulations of lung dendritic cells (DCs) in our models. We observed, during the NETs release phase, a recruitment of monocytic-derived DCs responsible for allergic sensitization. This recruitment was abrogated when NETS were inhibited. In conclusion, our study reveals how apparently unrelated environmental risk factors commonly shape immune responses, by recruiting particular neutrophils which release NETs, to promote asthma.

WS.A2.04.04
Alpha-Gal bound to lipids, but not to proteins, is able to cross the intestinal epithelium and might thus cause delayed allergic symptoms in meat allergic patients

P. Román-Carrasco[1]*, B. Lieder*[2]*, V. Somoza*[2]*, M. Ponce*[3]*, Z. Szépfalusi*[3]*, D. Martín*[4]*, W. Hemmer*[5]*, I. Swoboda*[1]*;*
[1]*Molecular Biotechnology Section, University of Applied Sciences, Vienna, Austria,* [2]*Department of Physiological Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria,* [3]*Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria,* [4]*Departamento de Producción y Caracterización de Nuevos Alimentos. Instituto de Investigación en Ciencias de la Alimentación (CIAL), Madrid, Spain,* [5]*FAZ-Floridsdorf Allergy Center, Vienna, Austria.*

The oligosaccharide galactose-α-1,3-galactose (α-Gal), present on mammalian proteins and lipids, causes an unusual delayed allergic reaction 3 to 6 hours after ingestion of mammalian meat, in individuals with IgE antibodies against α-Gal. To better understand the delayed onset of allergic symptoms and investigate whether protein-bound or lipid-bound α-Gal is responsible for them, we analyzed the capacity of α-Gal conjugated proteins and lipids to cross the intestinal epithelium. For this, extracts of proteins and lipids from cooked beef were prepared, subjected to *in vitro* simulated digestions and added to Caco-2 cells grown on permeable inserts. The presence of α-Gal was investigated in the basolateral medium by immunoblotting, thin-layer chromatography (TLC) with immunostaining and ELISA and its allergenic activity was analyzed in a basophil activation test. After addition of beef proteins on the apical side of Caco-2 cells, α-Gal containing peptides were not detected in the basolateral medium and those peptides that crossed the Caco-2 monolayer did not activate basophils from an α-Gal allergic patient. Instead, when Caco-2 cells were incubated with lipids extracted from beef, α-Gal was detected in the basolateral medium. Furthermore, these α-Gal lipids, were able to activate the basophils of an α-Gal allergic patient in a dose-dependent manner. We thus showed that only α-Gal carried on lipids, but not on proteins is able to cross the intestinal epithelium and trigger an allergic reaction. The slower digestion process of α-Gal conjugated lipids might explain the delay in the appearance of symptoms since it takes for dietary lipids a longer time than for proteins to reach the circulation.

WS.A2.04.05
Cytofast: A workflow for flow and mass cytometry data to discover group-related immune signatures

G. Beyrend*, K. Stam, T. Höllt, R. Arens;*
Leiden University Medical Center, LEIDEN, Netherlands.

Multi-parametric flow and mass cytometry allows exceptional high-resolution exploration of the cellular composition of the immune system. A large panel of tools have been developed to analyze the high-dimensional landscape of the data generated. Among them, Cytosplore, incorporating the HSNE algorithms, is highly suitable for multi-parametric cytometry analysis. However, this software focusses on visual exploration and does not provide means to quantify group-specific cell clusters or correlations with clinical observations. Here, we introduce an R-based workflow, called CyTOFastR, for downstream analysis of Cytosplore-processed cytometry data sets. CyTOFastR is generating visual representations to identify group-related immune cell clusters and to study the correlation of any clinical variable with the immune system composition. We apply our workflow on two previously published data sets, paired and non-paired, based on the modulation of the immune system upon a specific treatment. We discovered a new macrophage subset from a previous mass cytometry study, which decreased upon allogeneic tumor-binding IgG. Thus, our bioinformatic tool offers an automated time-efficient approach for comprehensive multi-parametric cytometry analysis to reveal group-related immune signatures.

WS.A2.04.06
A novel algorithm for analysis of the evolution of B-cell receptors repertoires using high-throughput sequencing data

A. S. Obraztsova[1]*, M. Shugay*[1,2,3,4]*, D. M. Chudakov*[1,2,3,4,5]*;*
[1]*Skolkovo Institute of Science and Technology, Moscow, Russian Federation,* [2]*Institute of Bioorganic Chemistry (RAS), Moscow, Russian Federation,* [3]*Pirogov Russian National Research Medical University, Moscow, Russian Federation,* [4]*Privolzhsky Research Medical University (PIMU), Moscow, Russian Federation,* [5]*Central European Institute of Technology (CEITEC), Praha, Czech Republic.*

One of the mechanisms that is critical for forming an efficient immune response is the affinity maturation of B-cells, a process during which somatic hypermutations (SHMs) are introduced into the Ig sequence followed by subsequent rounds of antigen-driven selection in germinal centers of lymph nodes. Recent advances in high-throughput sequencing techniques empowered a comprehensive study this phenomenon, but some technical hurdles still complicate the analysis of B-cell receptor repertoires. The present work is devoted to the solution of two problems. First, the background noise of PCR and sequencing errors that can interfere with SHM analysis can be eliminated using the molecular tagging technique and an extension of our previously published algorithm [1] to full-length Ig sequences. The second difficulty lies in the identification of SHMs that fall into the complementarity-determining region 3 (CDR3) of the Ig sequence that doesn't have any germline reference sequence. To solve the latter problem, we have developed a novel algorithm that can accurately identify and cluster CDR3 sequences that are far more similar than can be expected from random sampling of CDR3 repertoire. This approach allows us to accurately reconstruct the clonal trees of full-length Ig sequences present in the sample using the parsimony principle. The clonal lineage structure provides information about B-cell receptor repertoire evolution as well as making it possible to detect all SHMs located in CDR3. This work was supported by Russian Science Foundation (RSF) grant 14-14-00533.

WS.A3.01 Immunobiomarkers in autoimmunity and beyond

WS.A3.01.01
Dissecting IL-12p70 response variability in health and disease

C. Posseme[1]*, B. Charbit*[1]*, A. Llibre*[1]*, A. Bisiaux*[1]*, B. Piasecka*[1]*, E. Nemes*[2]*, T. Scriba*[2]*, L. Quintana-Murci*[1]*, S. Pol*[1]*, M. L. Albert*[3]*, D. Duffy*[1]*;*
[1]*Institut Pasteur, Paris, France,* [2]*SATVI, Cape Town, South Africa,* [3]*Genentech Inc, San Francisco, United States.*

Cytokines are essential regulators of immune responses and coordinate the response against pathogens. Therefore, they hold great potential as targets for new diagnostic and therapeutic strategies. IL-12p70 is a key heterodimer molecules for driving Th1 immune responses. Despite its role in combating infection we have observed highly variable IL-12p70 responses in healthy individuals, as well as in patients infected with tuberculosis (TB) and chronic hepatitis C (HCV). We hypothesize that dissecting this variance in a healthy population will provide new insights into disease pathogenesis. Following LPS stimulation of whole blood from 1,000 donors of the Milieu Interieur cohort, 28% of healthy donors failed to secrete IL-12p70, but its production was restored with IFNa co-stimulation. However, in HCV and TB patients, greater than 50% of the patients did not secrete IL-12p70 following LPS stimulation and only a subgroup of patients were able to restore IL-12p70 secretion upon co-stimulation with IFNa.

Utilizing the Milieu Interieur cohort we have further investigated the factors behind this variability. Having identified by flow cytometry that monocytes and dendritic cells are the main IL-12p70 producers in whole blood, we confirmed that cellular differences were not responsible for the variability in healthy donors. In contrast gene set enrichment analysis revealed significant IFNg response differences between the two phenotypes under LPS stimulation, and protein Quantitative Trait Loci analysis revealed a genetic association with IL-12p70 responses. These results will allow to investigate the intracellular mechanisms by blocking specific pathways and to restore them in chronically infected HCV or TB patients.

WS.A3.01.02

New protein array technology identifies rituximab treated non responder rheumatoid arthritis patients are generating a new autoantibody repertoire

*Z. Konthur[1], M. Wiemkes[2], T. Häupl[2], G. Burmester[2], **K. Skriner**[2];*
[1]Max Planck Institute of Colloids and Interfaces, Berlin, Germany, [2]Charité University Medicine, Berlin, Germany.

Background: Rituximab (RTX) has shown clinical efficacy in cancer and autoimmune diseases but up to 40 % of RTX treated rheumatoid arthritis (RA) patients are poor responders (ref:1) and the commonly used RA biomarkers (*RF/ACPA)* are poor predictors for therapy response. **Methods:** Screening of RA sera was conducted on 37.830 unique human protein marcoarrays (http://www.engine-gmbh.de) with sera taken before and 24 weeks after treatment. Autoantibody response of different immunoglobulin classes IgD, IgA, and IgG was recorded and bioinformatically evaluated. Response was determined according to DAS28 criteria. **Results:** In the cohort of 26 patients 1292 different autoantigens (IgD,IgA,IgG) were detected. Using protein array we investigated clusters of autoantigen responses that disappeared or developed during RTX treatment of RA patients. Post treatment developing responses against new autoantigens can be correlated to mRNA tissue expression data. RA autoantigenic patterns before and 6 month after RTX treatment were patient-specific. RTX reduced the repertoire of autoantibodies after 24 weeks of treatment in the tested RA patient cohort on average by 60%. RA patients which do not respond are generating on average 63% new autoantibodies. In good responders to RTX only 5,5% (+/-3%) new autoantibodies can be detected. The IgA and IgG autoantibody repertoire in the serum after 24 weeks of RTX treatment is reduced (IgA: 41%, IgG :31%) in good responders whereas it is increased (IgA: 1,3%, IgG: 24%) in non responders to RTX. **Conclusions:** Non responders to RTX change their autoantibody repertoire directed against new but patient specific antigens. **References:** 1:AnnRheumDis. 2005 Feb;64(2):246-52.

WS.A3.01.03

Imbalance of naïve and memory T cells in peripheral lymphocyte subpopulations at onset of type 1 diabetes

*A. Teniente-Serra[1], E. Pizarro[2], **C. Esteve-Cols**[1], M. Julian[2], M. Fernández[3], E. Martínez-Cáceres[1];*
[1]Germans Trias i Pujol University Hospital, Badalona, Spain, [2]Hospital de Mataró, Mataró, Spain, [3], Germans Trias i Pujol Research Institute (IGTP), Campus Can Ruti, Badalona, Spain.

Introduction: Type 1 diabetes (T1D) is an autoimmune disorder characterized by destruction of pancreatic beta cells resulting in insulin dependency. Changes in T and B cell subpopulations in peripheral blood of T1D patients have been described, but a comprehensive multiparametric flow cytometric analysis is still lacking.
Aim: To identify changes in peripheral blood T- and B- cell compartments in patients at onset of T1D.
Material and methods: CD4+ and CD8+ T cells (including naïve, central memory, effector memory and terminally differentiated effector (TEMRA), Th17 and Tregs) and B cells subsets (naïve, unswitched memory, switched memory and transitional B cells) were analyzed in peripheral blood of T1D patients at onset (n=26) and healthy donors (HD; n=40) using multiparametric flow cytometry.
Results: A decrease in the percentage of early and late effector memory CD4+ and CD8+ T cells (TCD4+: p=0.001 and p<0.001, TCD8+: p=0.046 and p<0.001), TEMRA CD4+ and CD8+ cells (p=0.003 and p=0.004, respectively) was found. In contrast, the percentage of naïve CD4+ T cells (p=0.010), and percentage and absolute counts of naïve CD8+ T cells (p<0.001 and p=0.001) were increased in peripheral blood of T1D patients compared with HD. Moreover, an increase in percentage of total B cells and transitional B cells was observed in patients compared with HD (p=0.015 and p=0.006, respectively). No changes were found either in Tregs or in Th17 subpopulations.
Conclusion: The observed changes in the percentage and/or absolute number of lymphocyte subpopulations support that effector cells migrate to the pancreas participating in the autoimmune response.

WS.A3.01.04

Glycolytic T cell metabolism drives faster disease progressionin progressive multiple sclerosis patients

***S. DE BIASI**, E. Bianchini, M. Nasi, L. Gibellini, S. Pecorini, D. Lo Tartaro, A. Simone, D. Ferraro, F. Vitetta, P. Sola, A. Cossarizza, M. Pinti;*
UNIVERSITY OF MODENA AND REGGIO EMILIA, MODENA, Italy.

Introduction. Inflammation and neurodegeneration sustain disease progression in both primary progressive (PP) and secondary progressive (SP) form of multiple sclerosis (MS). PP and SP forms present different symptoms, but the inflammatory status and the neurodegenerative process are indistinguishable. Differences in CD4 T-cell biology identify SP patients having different rates of progression, emphasizing an association between the systemic immune activation and disease progression. However, the mechanisms responsible of such impairment are still unknown. **Aim.**T cell activation is accompanied by a switch from a metabolism mainly based upon mitochondrial respiration to a metabolism where glycolytic flux is prevalent. Hence, we investigated the metabolic changes and mitochondria (mt) functionality of T cell subpopulations in 46 progressive MS patients, to clarify if PP form is part of the disease spectrum, or a distinct entity. **Results.** When compared to SP patients, T cells from PP displayed a senescent phenotype (low proliferation, increase of terminally differentiated/exhausted cells), lower mt mass, membrane potential and respiration, a more marked down-regulation of transcription factors supporting respiration. This is counterbalanced by higher mTOR activity and higher expression of glycolytic-supporting genes. Higher levels of lactate were found in plasma of PP patients. **Conclusion.**SP and PP patients displayed differences from the phenotypic and metabolic point of view. These differences, driven by deficit or abnormalities in the metabolism of immune cells, can contribute to neurodegeneration and chronic level of inflammation in PP and SP patients, determining a faster disease progression in PP.

WS.A3.01.05

Measurement of serum infliximab levels and detection of free and bound anti-infliximab antibodies in patients with rheumatoid arthritis

***C. Hermanrud**[1], M. Ryner[1], R. Pullerits[2,3], K. Hambardzumyan[4], N. Vivar Pomiano[4], P. Marits[5], I. Gjertsson[2], S. Saevarsdottir[4], A. Fogdell-Hahn[1];*
[1]Clinical Neuroimmunology, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden, [2]Department of Rheumatology and Inflammation Research, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden, [3]Department of Clinical Immunology, Sahlgrenska University Hospital, Gothenburg, Sweden, [4]Rheumatology Unit, Department of Medicine, Solna, Karolinska Institutet, Stockholm, Sweden, [5]Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden.

Introduction: Tumor necrosis factor-alpha (TNF-α) inhibitors are used to treat symptoms of rheumatoid arthritis (RA). Low drug levels correlate well with the presence of anti-drug antibodies (ADA) and are likely to result in a poor clinical outcome. Levels of free TNF-α inhibitor and ADA are detected with an in-house validated ELISA used in clinical routine at Karolinska University Hospital. However, false negative results can occur due to the formation of drug/ADA immune complexes and that samples with a drug level >0.2 µg/mL should not be screened for ADA with ELISA because of the assays low drug tolerance. **Methods:** Drug levels and ADA were studied with three methods; free ADA (ELISA), neutralizing ADA (bioassay, iLite) and total ADA (precipitation and acid dissociation assay, PandA). Three RA cohorts were included; SWEFOT (prospective, n=101) Karolinska University Hospital, and REALlife with patients from Karolinska University Hospital, (cross-sectional, n=272) and Sahlgrenska University Hospital (prospective, n=42). **Results:** The majority of patients (SWEFOT 46%; REALlife 70%) had suboptimal TNF-α inhibitor serum concentration (<3 µg/mL), and around 24% had an optimal drug concentration (3-7 µg/mL). As expected, low serum TNF-α inhibitor levels (<0.2 µg/mL) correlated with ADA, of which 66% were neutralizing. Moreover, PandA measured free and bound ADA reactivity in 24% of tested serum samples that had detectable TNF-α inhibitor levels (0.2-7 µg/mL). **Conclusion:** There was a clear difference in the drug level between the clinical trial cohort and the real-life situation, showing that expected ADA prevalence might need to be adjusted in a routine clinical setting.

WS.A3.01.06

Imaging flow cytometry enhances the detection of small particles and rare events enabling emerging applications in immunology and oncology

***P. Rhein**[1], A. Goergens[2], B. Giebel[3], S. Groenefeld-Krentz[4], C. Eckert[4];*
[1]Merck KGaA, Darmstadt, Germany, [2]Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden, [3]Institute of Transfusion Medicine, University Hospital Essen, Essen, Germany, [4]Pediatric Oncology/Hematology, Charité-Universitätsmedizin, Berlin, Germany.

Detection of small particles and rare events by flow cytometry is often hampered by the limited amount of information that can be gathered from light scatter signals and fluorescence. Small particles like extracellular vesicles (EVs) have recently gained increased interest as they are physiologically and diagnostically relevant. The small but variable size and abundance made analysis of single EV difficult on traditional flow cytometers.For rare events detection discrimination of relevant events and artifacts is absolutely mandatory and requires complex and challenging experimental design for traditional flow cytometers. One example are circulating tumor cells (CTCs), released into the bloodstream from primary and metastatic cancers and tumor cells persisting during therapy (minimal residual disease, MRD) that have important prognostic and therapeutic implications and are valuable tools for understanding tumor biology. The ImageStream® is a multispectral imaging flow cytometer that helps to overcome these obstacles by combining the statistical power of flow cytometry with imaging content of microscopy in one system. We are now able to characterize EVs including exosomes (70 nm - 160 nm in diameter), microvesicles and apoptotioc bodies at the single EV level. Moreover, the study shows the significance of the imagery for the detection of CTCs by preventing the misclassification of EpCAM+ CD45+ events as leukocytes and the undercounting of CTC doublets as single cells. And we demonstrate the feasibility of fluorescence in situ hybridization (FISH) in flow which can contribute to significantly improve the detection and functional analysis of biological rare events.

WS.A3.02 Biomarkers of adaptive immunity

WS.A3.02.01
Rapid identification and isolation of functional antigen-specific CD8⁺ T cells by staining of activated integrins

S. Dimitrov[1], *C. Gouttefangeas*[1], *L. Besedovsky*[1], *A. T. Jensen*[2], *A. Chandrand*[1], *E. Rusch*[1], *R. Businger*[1], *M. Schindler*[1], *T. Lange*[3], *J. Born*[1], *H. Rammensee*[1];
[1]*University of Tübingen, Tübingen, Germany,* [2]*University of Copenhagen, Copenhagen, Denmark,* [3]*University of Lübeck, Lübeck, Germany.*

Immediate changes in the conformation and clustering of β_2-integrins upon T-cell receptor stimulation is critical for the strong adhesion of antigen-specific T cells to their targets and the downstream execution of T-cell effector functions. Integrin activation may therefore be used for the rapid identification of functional T cells. We present a novel, simple, and sensitive flow cytometry-based assay to assess antigen-specific T cells using fluorescent intercellular adhesion molecule (ICAM)-1 multimers that specifically bind to activated β_2-integrins. The method is compatible with surface and intracellular staining; it is applicable for monitoring of a broad range of virus-, tumor- and vaccine-specific CD8⁺ T cells, and for isolating viable antigen-reacting cells. ICAM-1 binding correlates with peptide-MHC multimer binding, but, notably, it identifies the fraction of antigen-specific CD8⁺ T cells with immediate and high functional capability, i.e., expressing high levels of cytotoxic markers and cytokines. Compared to the currently available methods, staining of activated β_2-integrins presents the unique advantage of requiring activation times of only several minutes, therefore delivering functional information nearly reflecting the *in vivo* situation. Hence, the ICAM-1 assay is utmost suitable for rapid and precise monitoring of functional antigen-specific T-cell responses including for patient samples in various clinical settings.

WS.A3.02.02
Epigenetic signatures of human T helper cell subsets

A. Ntalli[1], *S. Kumar*[2], *L. Maggi*[3], *F. Annunziato*[3], *C. Zinser*[4], *M. Beckstette*[1], *L. Graca*[2], *S. Floess*[1], *J. Huehn*[1];
[1]*Department of Experimental Immunology, Helmholtz Centre for Infection Research, Braunschweig, Germany,* [2]*Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal,* [3]*Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy,* [4]*Genomatix Software GmbH, Muenchen, Germany.*

Naive CD4⁺ T cells are highly plastic cells that - upon activation - can differentiate into various T helper (Th) cell fates characterized by the expression of specific transcription factors and effector cytokines. Their expression can be stabilized by epigenetic mechanisms including DNA methylation. So far, our knowledge about the link between DNA methylation and T helper cell differentiation processes is fragmentary. After the identification of an epigenetic Th17 signature suitable for the characterization of murine cells, we wanted to extend this knowledge to the human epigenome by performing a whole-genome bisulfite sequencing analysis of *ex vivo* isolated human naïve T cells and selected clones of Th1, non-classic Th1 and Th17 cells. The comparison of the different methylomes allowed for the detection of differentially methylated regions (DMRs) mainly located within introns, followed by promoter regions and exons. In accordance with literature, several DMRs were identified within lineage-specific loci like *IFNG*, *TBX21*, *IL17A* and *RORC2*. Additionally, we identified novel DMRs and cloned them into CpG-free luciferase reporter plasmids to study their transcriptional activity. Luciferase reporter assays in primary human CD4⁺ T cells revealed that DMRs within *SLAMF8* and *SRFS7* mediate transcriptional activity in a methylation-dependent fashion. In addition, we performed expression studies using *ex vivo* isolated T naïve, Th1 and Th17 helper cells to identify transcription factors that participate in transcriptional regulation via the DMRs. Thereby, we might be able to define lineage-specific regulatory modules involved in human T helper cell differentiation.

WS.A3.02.03
The cold shock protein YB-1 (Y-box binding protein 1) promotes survival of CD4⁺ T-lymphocytes

S. Meltendorf[1], *S. Gieseler-Halbach*[1], *J. Handschuh*[2], *M. Pierau*[1], *A. Arra*[1], *P. R. Mertens*[3], *U. Thomas*[4], *M. C. Brunner-Weinzierl*[1];
[1]*Department of Experimental Paediatrics, Magdeburg, Germany,* [2]*Leibniz Institute for Neurobiology, Magdeburg, Germany,* [3]*Department of Nephrology and Hypertension, Diabetes and Endocrinology, Magdeburg, Germany,* [4]*Departmente of Neurochemistry and Molecular Biology, Leibniz Institute for Neurobiology, Magdeburg, Germany.*

The cold shock protein YB-1 is highly expressed in tumours, such as breast cancer, and associated with hyper proliferation and resistance against apoptosis. Enhanced YB-1 expression at the transcription and nuclear protein levels have been shown to correlate with poor prognosis and resistance to chemotherapy for tumour patients. However, the role of YB-1 in T-lymphocytes is not understood so far. Human CD4⁺ T-lymphocytes isolated from PBMCs were stimulated with anti-CD3/anti-CD28 coupled beads. YB-1-signaling was manipulated by overexpression of YB-1^{wt} using lentiviral transduction of FuGW-GFP-constructs or reduction of YB-1 expression using specific YB-1shRNA. Apoptosis measurement was carried out six to eight days after initial stimulation and analysed. Expression of pro-and anti-apoptotic molecules was monitored by flow cytometry, western blot, and real-time PCR. Transduction of GFP-YB-1^{wt} constructs and mutant variants thereof into primary human CD4⁺ T-cells yielded around 40% GFP positive cells. We observed that YB-1 overexpression enhanced survival by 60% compared to the control. Additionally, the percentage of surviving cells was increased with specific inhibitors (QVD) for apoptosis in empty vector-transduced cells but not in YB-1^{wt} overexpressing cells. Furthermore, forced reductions of YB-1 with YB-1shRNA lead to an reduced survival in T-Lymphocytes. A substantial reduction in the mRNA and protein levels of anti-apoptotic molecule Bcl-xl following YB-1 knock-down clearly indicated the impact of YB-1 in regulating the T cell apoptosis. Thus, YB-1 tightly controls and promotes survival in T-cells.

WS.A3.02.04
Tissue resident memory T cells as a progressive multifocal leukoencephalopathy quick biomarker

A. Tejeda Velarde[1], *E. Rodríguez Martín*[1], *L. Costa-Frossard*[1], *Y. Aladro*[2], *S. Sainz de la Maza*[1], *S. Medina*[1], *N. Villarrubia*[1], *E. Monreal*[1], *E. Roldán*[1], *J. Álvarez-Cermeño*[1], *L. Villar*[1];
[1]*Ramón y Cajal University Hospital, Madrid, Spain,* [2]*Getafe University Hospital, Madrid, Spain.*

Introduction: Progressive multifocal leukoencephalopathy (PML) is a serious side effect associated with immune system modifying drugs in multiple sclerosis (MS). PML is caused by the opportunistic infection by John Cunningham virus and its early diagnosis is crucial for patient's survival. Tissue resident memory T cells (T_{RM}) are important during viral infection, and they can be detected in cerebrospinal fluid (CSF) by flow cytometry in only one hour. We aimed to explore if PML patients show an increase in T_{RM} and, in addition, to explore other CD4 and CD8 T cell subsets.
Patients and methods: We included 86 MS patients: three suffered PML (PML+) and 83 did not (PML-). CSF T cell subsets were explored by flow cytometry in a FACSCanto II cytometer. Results were expressed as percentage of CD45 mononuclear cells. Mann-Whitney U test was applied for comparison between groups.
Results: PML+ showed lower CD4 (p=0.0062), naïve CD4 (p=0.0111) and central memory CD4 (p=0.0440) compared to PML-. By contrast, in PML+ we observed a higher percentage of CD8 (p=0.0086) and terminally differentiated CD8 (p=0.0219) compared to PML-. However, the most clear difference was found in T_{RM} CD8 that were considerably higher in PML+ than in PML- (Respectively, 11.57%±5.17 vs 0.46%±0.05 [mean ± standard error], p=0.0081).
Conclusions: Although these findings should be validated in a larger cohort, our data show that PML induces a significant change in CSF T cell subsets of MS patients and strongly suggest that T_{RM} detection could be a good tool for an early PML diagnosis.

WS.A3.02.05
T3SS components as the biomarkers of humoral immune response elicited by live bacterial vaccine in humans

V. A. Feodorova[1,2], *A. M. Lyapina*[1], *M. A. Khizhnyakova*[1], *S. S. Zaitsev*[1], *L. V. Sayapina*[3], *M. V. Telepnev*[4], *O. V. Ulianova*[1], *E. P. Lyapina*[5], *S. S. Ulyanov*[6], *V. L. Motin*[4];
[1]*Federal Research Center for Virology and Microbiology, Branch in Saratov, Saratov, Russian Federation,* [2]*Saratov State Agrarian University named after N.I. Vavilov, Saratov, Russian Federation,* [3]*Scientific Center on Expertise of Medical Application Products, Moscow, Russian Federation,* [4]*University of Texas Medical Branch, Galveston, United States,* [5]*Saratov State Medical University named after V.I. Razumovsky, Saratov, Russian Federation,* [6]*Saratov State National Research University, Saratov, Russian Federation.*

Type III Secretion System (T3SS), a system of proteins known as a powerful bacterial tool for subverting host immune response by Gram-negative pathogens. T3SS components as the key virulence factors are an attractive target for both vaccine and immunological markers development. In this study, using immunoblot technique and highly pure recombinant antigens, we investigated the potential of T3SS components, namely LcrV, YopM and YopE, to serve as the markers of humoral immune response in humans multiply vaccinated with live plague vaccine LPV, an attenuated *Y. pestis* EV strain line NIIEG possessing T3SS (n = 34). Sera from healthy naïve donors (n = 17) were used as a control. We found that anti-YopE antibodies, but not anti-YopM or anti-LcrV, were highly specific for the vaccinated group ($p<0.05$). Interestingly, humoral response to both YopE and YopM was likely short-circuiting since it correlated negatively with the post immunization period ($p<0.05$). There was an inverse association between the number of LPV injections and positive responses to YopM ($p<0.05$). No analogous relationships were observed for LcrV. Thus, we have shown that T3SS components may be promising for the development of plague humoral immunity markers. This work was supported by the RFBR #18-016-00159.

WS.A3.02.06

Skin-homing human CD8+ T cells preferentially express GPI-anchored peptidase inhibitor 16 (PI16), an inhibitor of cathepsin K

N. Lupsa[1,2], *B. Érsek*[1,3], *A. Bencsik*[1], *A. Horváth*[1], *E. Lajkó*[1], *Á. Oszvald*[1], *Z. Wiener*[1], *P. Reményi*[4], *G. Mikala*[4], *T. Masszi*[4,5], *E. Buzás*[1,2], *Z. Pós*[1];
[1]*Semmelweis University, Dept. of Genetics, Cell and Immunbiology, Budapest, Hungary,* [2]*Hungarian Academy of Sciences - Semmelweis University Immunoproteogenomics Extracellular Vesicle Research Group, Budapest, Hungary,* [3]*Office for Research Groups Attached to Universities and Other Institutions of the Hungarian Academy of Sciences, Budapest, Hungary,* [4]*Department of Hematology and Stem Cell Transplantation of the St. Istvan and Saint Laszlo Hospital, Budapest, Hungary,* [5]*3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary.*

This study sought to identify novel markers of skin- and gut-homing CD8+ T cells by analyzing them in acute graft versus host disease (aGvHD), typically involving CD8+ T cell-mediated organ damage of the skin and gut.
Patients undergoing allogeneic hematopoietic stem cell transplantation were assigned to groups developing cutaneous aGVHD, gastrointestinal aGvHD, both, or none, and their sorted skin-homing (CD8+/CLA+), gut-homing (CD8+/integrinβ7+), and reference (CD8+/CLA-/integrinβ7-) T cells were compared. Microarray analysis, Q-PCR and flow cytometry disclosed increased expression of peptidase inhibitor 16 (PI16) in skin-homing CD8+ T cells. PI16 was expressed by CD8+ T cells regardless of the organ(s) affected by aGvHD, aGvHD as such, and remained associated to skin-homing T cells in healthy blood donors, too. PI16 was not observed on CLA+ leukocytes other than T cells, and was restricted to the non-naïve CD45RO+ compartment. Induction of PI16 expression was independent of vitamin D3, remained unaffected by retinoic acid, or by co-culture with human skin and intestinal organoids. PI16 was confined to the plasma membrane, appeared GPI-anchored, and became lost upon re-stimulation of circulating skin-homing T cells. Loss of PI16 occurred by rapid downregulation of PI16 transcription, not by PLC- or ACE-mediated shedding, or by recycling from the plasma membrane. Inhibitor screening and pull-down experiments confirmed that PI16 has low affinity, if any, to most skin proteases, but inhibits cathepsin-K.
These data demonstrate robust PI16 expression in skin-homing CD8+ T cells, and raise the possibility that PI16 may inhibit an inflammatory skin protease until cutaneous CD8+ T cell activation.

WS.A3.03 Immune markers in malignancies

WS.A3.03.01

NSCLC patients not responding to nivolumab show lowered frequency of co-stimulatory receptor-deficient CD8 T cells

A. Kunert*, E. A. Basak, D. P. Hurkmans, Y. Klaver, M. van Brakel, A. Oostvogels, C. H. Lamers, S. Bins, S. L. Koolen, A. A. van der Veldt, S. Sleijfer, R. H. Mathijssen, J. G. Aerts, R. Debets;*
Erasmus MC Cancer Institute, Rotterdam, Netherlands.

Checkpoint inhibitors have become standard care of treatment for non-small cell lung cancer (NSCLC). As only a limited fraction of patients experience durable clinical benefit, there is an urgent need to identify patients with early progressive disease. In the current discovery study, we have analyzed peripheral blood samples of 71 NSCLC patients treated with 2nd line nivolumab prior and throughout therapy with multiplex flow cytometry enumerating 20 immune cell subsets, and assessing frequencies of T cells using over 300 combinations of markers. Best overall response was assessed according to RECIST within 90 days from start of treatment. We discovered that patients with progressive and stable disease (PD and SD) displayed on average a 2-fold decrease in numbers of CD8 T cells prior to and throughout therapy, while patients showing partial response (PR) showed levels similar to those of healthy individuals with about 560 cells/µl. Analysis of T cell subsets expressing variable numbers of co-stimulatory or co-inhibitory receptors revealed that this reduction was accompanied by an enhanced expression co-stimulatory receptors in CD8 T cells of PD patients, while in PR patients they displayed a more exhausted phenotype. Interestingly, upon treatment with nivolumab, PD patients also showed a drop in the frequency of CD4 T cells expressing PD1 and BTLA, which may represent antigen-experienced helper T cells. Our study demonstrates that numbers of CD8 T cells as well as frequencies of CD8 and CD4 T cells with defined co-signaling signatures in peripheral blood are associated with response to nivolumab in NSCLC patients.

WS.A3.03.02

Circulating CD4 senescent T cells stratify clinical responses to PD-L1/PD-1 immune checkpoint inhibitors in NSCLC

M. Zuazo[1]*, H. Arasanz*[1]*, M. Gato*[1]*, G. Fernández*[2]*, R. Vera*[2]*, G. Kochan*[1]*, D. Escors*[1,3]*;*
[1]*Navarrabiomed, Pamplona, Spain,* [2]*Complejo Hospitalario de Navarra, Pamplona, Spain,* [3]*University College London, London, United Kingdom.*

Background: PD-L1/PD-1 blockade immunotherapies are demonstrating promising clinical outcomes in different neoplasms, although response rates are low and no accurate biomarkers of response have been discovered. Senescent T cells (Tsens) accumulate with age and comprise a large pool of antigen-specific T cells. Here, the impact of PD-L1/PD-1 blockade immunotherapy on systemic Tsens was assessed. **Methods:** A prospective small-scale study was conducted in 33 non-small cell lung cancer (NSCLC) patients treated with PD-L1/PD-1 immune checkpoint inhibitors. Baseline Tsens and their dynamic changes during treatment were quantified from peripheral blood samples and correlated with clinical efficacy based on RECIST criteria. **Results:** In our cohort study, patients with Tsen baseline values below 40% (negative baseline profile accounting to 52% of patients) had an ORR of 0% and 6 weeks PFS, in contrast to the remaining patients with a 37,5% response rate. Two main dynamic patterns of responses were identified. Increase in Tsens after the first cycle of therapy was always associated to progression (pattern 1, Tsen "burst"), while decrease (pattern 2) significantly correlated with responders. Hyperprogression was found in patients with a negative baseline profile and highly significant systemic CD4 Tsen bursts. **Conclusions:** Quantification of CD4 Tsens from routine blood samples provides an accurate predictive biomarker of responses with highly significant stratification power in NSCLC. Patients with a negative Tsen baseline profile did not respond to PD-L1/PD-1 immune checkpoint inhibitors or exhibited hyperprogressive disease. Enrolment of these patients for PD-L1/PD-1 blockade monotherapy should be avoided if other therapies are available.

WS.A3.03.03

CD103 tumor Infiltrating lymphocytes as a prognostic factor in colon cancer patients

s. M. talhouni*, J. Ramage;*
Nottingham City Hospital, Nottingham, United Kingdom.

Background: The primary site of colorectal cancer can either be in the colon or the rectum. Due to anatomic continuity of the colon into the rectum, cancers affecting these organs have historically been considered equivalent. However, recent studies have shown increased mutational burden and microsatellite instability in right-sided colon tumours versus left-sided colon and rectal tumours which may affect patient survival. T cells infiltrating tumours have shown a role as prognostic biomarker in colorectal cancer. However, recently CD103 a marker for non-circulating tissue resident memory (TRM) T cells have shown a stronger correlation with survival than CD3 or CD8 T cells in ovarian, breast and lung cancer. In this study, we investigated the association between CD103 TILs and the overall survival of right-sided colon, left-sided colon, and rectal cancer patients. Immunohistochemistry staining was conducted on validated colorectal TMA sections using a monoclonal antibody to CD103, its impact in terms of survival (in months) and clinic-pathological variables was determined **Results:** CD103 TILs were present in both colon and rectal cancer patients. However, Intraepithelial CD103 was associated with better overall survival in right colon cancer patients (p=0.01) in comparison to left sided colon and rectal cancer patients (p=0.665 and 0.818, respectively). **Conclusion:** We hypothesize that increased mutational rates in the right sided colon tumours improved the prognostic role of CD103+ antigen presenting TRM cells in comparison to the left colon/rectal tumours.

WS.A3.03.04

Filgrastim enhances T-cell clearance by anti-thymocyte globulin exposure after unrelated cord blood transplantation

C. de Koning[1]*, J. A. Gabelich*[1]*, J. Langenhorst*[1]*, R. Admiraal*[1]*, J. Kuball*[1]*, J. J. Boelens*[2,1]*, S. Nierkens*[1]*;*
[1]*University Medical Centre Utrecht, Utrecht, Netherlands,* [2]*Wilhemina Children's Hospital, Utrecht, Netherlands.*

Residual anti-thymocyte globulin (ATG; Thymoglobulin) exposure after allogeneic hematopoietic (stem) cell transplantation (HCT) delays CD4+ T-cell immune reconstitution (CD4+ IR), subsequently increasing morbidity and mortality. This effect seems particularly present after cord blood transplantation (CBT) compared bone marrow transplantation (BMT). The reason for this is currently unknown. We investigated the effect of active-ATG exposure on CD4+ IR after BMT and CBT in 275 patients (CBT; n=155, BMT; n=120, median age: 7.8 years; range 0.16-19.2) receiving their first allogeneic HCT between Jan-2008 and Sept-2016. Multivariate log-rank tests (with correction for covariates) revealed that CD4+ IR was faster after CBT than after BMT with <10 active-ATG*day/mL (p=0.018) residual exposure. In contrast, >10 active-ATG*day/mL exposure severely impaired CD4+ IR after CBT (p<0.001), but not after BMT (p=0.74). To decipher these differences, we performed ATG-binding and -cytotoxicity experiments using CB- and BM-graft-derived T-cell subsets, B-, NK-cells, and monocytes. No differences were observed. Nevertheless, a major covariate in our cohort was Filgrastim treatment (only given after CBT). We found that Filgrastim (G-CSF) exposure highly increased neutrophil-mediated ATG-cytotoxicity, by 40-fold (0.5 vs. 20%, p=0.002), which explained the enhanced T-cell clearance after CBT. These findings imply revision of the use (and/or timing) of G-CSF in patients with residual ATG exposure.

WS.A3.03.05

Next-generation antigen receptor sequencing of paired diagnosis and relapse samples of B-cell acute lymphoblastic leukemia: an algorithm for Minimal Residual Disease target selection

*P. Theunissen[1], M. de Bie[1], D. van Zessen[1], V. de Haas[2], A. P. Stubbs[3], **V. H. J. van der Velden**[1];*
[1]Department of Immunology, Erasmus MC, Rotterdam, Netherlands, [2]DCOG, The Hague, Netherlands, [3]Department of Bioinformatics, Erasmus MC, Rotterdam, Netherlands.

Antigen receptor gene rearrangements are frequently applied as molecular targets for detection of minimal residual disease (MRD) in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) patients. Since such targets may however be lost at relapse, appropriate selection of antigen receptor genes as MRD-PCR target is critical. Recently, next-generation sequencing (NGS) - much more sensitive and quantitative than classical PCR-heteroduplex approaches - has been introduced for identification of MRD-PCR targets. In this study, we evaluated 42 paired diagnosis-relapse samples by NGS (IGH, IGK, TRG, TRD, TRB) to design an algorithm for selection of antigen receptor gene rearrangements which are most likely to remain stable at relapse. Overall, only 393 out of 1446 (27%) clonal rearrangements were stable between diagnosis and relapse. If only index clones with a frequency >5% at diagnosis were taken into account, this number increased to 65%; including only index clones with an absolute read count >10000, indicating truly major clones, further increased the stability to 84%. Over 90% of index clones at relapse were also present as index clone at diagnosis. Together, our data provide detailed information about the stability of antigen receptor gene rearrangements, based on which we propose an algorithm for selecting stable PCR targets for MRD monitoring, which should enable successful detection of relapse in >95% of BCP-ALL patients.

WS.A3.03.06

Plasma-derived exosomes in head and neck squamous cell carcinoma patients as potential biomarkers of response to immune therapies

***M. Theodoraki**[1], T. Hoffmann[1], R. Ferris[2], T. Whiteside[3];*
[1]Department of Otorhinolaryngology, Head and Neck Surgery, University of Ulm, Germany, Ulm, Germany, [2]Department of Otolaryngology-Head and Neck Surgery, University of Pittsburgh, Pittsburgh, PA, USA, Pittsburgh, United States, [3]Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA, Pittsburgh, United States.

Background: Circulating exosomes play a key role in immune suppression and disease progression. To evaluate their role as biomarkers of response to immunotherapy, we monitored changes in the cargo of exosomes from plasma of patients with head and neck squamous cell carcinoma (HNSCC) treated with Cetuximab, Ipilimumab and IMRT. **Methods:** Patients (n=18) with advanced HNSCC enrolled in the phase I clinical trial (NCT01935921) donated plasma at baseline and during (week 5, 14) immunotherapy. Exosomes were isolated by size exclusion chromatography and were separated into T cell-derived and non-T cell-derived exosomes by immunoaffinity capture. On-bead flow cytometry was used for detection of CTLA-4, PD-L1 and CD15s (Treg marker) on exosomes. To immunocapture tumor-derived exosomes (TEX), a microarray containing 4 antibodies specific for antigens overexpressed on HNSCC was used. Results were correlated to patients' outcome. **Results:** All patients had high TEX levels at baseline with a decrease at week 5. However, in patients who recurred (n=5), TEX levels increased at week 14; in contrast, TEX levels in 13 patients responding to therapy remained low. PD-L1 levels in CD3(-) exosomes (enriched in TEX) were elevated at week 5 in patients with recurrence but significantly decreased in responders to therapy. The responders had significantly higher levels of CTLA4 in exosomes at baseline than non-responders. However, a drastic decrease of CTLA4 occurred during therapy. Levels of CD15s were elevated at week 5 only in non-responders. **Conclusions**: Exosomes in plasma of cancer patients treated with immune therapies may serve as biomarkers of early response to treatment.

WS.A4.01 Germinal center reactions

WS.A4.01.01

Follicular dendritic cells originate from subepithelial mesenchymal cells in Peyer's patches

***A. Prados**[1], V. Koliaraki[1], G. Kollias[1,2];*
[1]BSRC Alexander Fleming, Vari, Greece, [2]National and Kapodistrian University of Athens, Athens, Greece.

Peyer's patches (PPs) are lymphoid organs that are located in the small intestine and play an important role in gut immunity. B and T lymphocytes are the main cell populations, which are segregated into different lymphoid areas, driven by the presence of two major mesenchymal populations: fibroblastic reticular cells in the T cell area and follicular dendritic cells (FDCs) in the B cell area. Our group has previously shown that Collagen VI (ColVI)-Cre mice are a useful new tool for PP FDC analysis. Here, we used multicolor fate mapping systems in combination with confocal and light sheet fluorescent microscopy, to dissect the ontogeny and dynamics of this cell population. Analysis of adult PP from ColVI-Cre Confetti mice revealed the presence of monocolored cell columns connecting subepithelial mesenchymal cells and FDC networks, pointing towards an ontogenetic relation between them. To discern the directionality of this relation, we studied PP organogenesis. At embryonic day 18.5 ColVI-Cre$^+$ cells appeared as a single cell layer underneath the epithelium. During the first week of life, these cells proliferated, migrated into the muscle layer and differentiated into FDCs. Interestingly, this migration/differentiation process was lymphotoxin β receptor (LtβR) dependent, since ColVI-Cre$^+$ cells in the LtβR$^{ColVI\text{-}CreKO}$ adult mice were restricted to a single cell layer under the epithelium. In contrast, specific deletion of Tnf receptor 1 (TnfR1) in ColVI-Cre$^+$ cells allowed their migration but blocked their differentiation into FDCs. To summarize, we demonstrate that during PP development, FDCs arise from subepithelial mesenchymal cells in a LtβR/TnfR1 dependent manner.

WS.A4.01.02

Novel branched proximity hybridization assay to quantify nanoscale protein-protein interaction

*S. Zheng[1], M. Mitterer[2], M. Reth[1], M. Cavallari[1], **J. YANG**[1];*
[1]Institute of Biology III, Freiburg, Germany, [2]Max-Planck-Institute of Immunobiology and Epigenetics, Freiburg, Germany.

Recent studies suggest that membrane proteins are pre-clustered and highly organized at nanometer (nm) distances. This cell surface nanoscale protein organization plays an essential role for receptor activation and signaling in both healthy and neoplastic cells. Duo the current technical limitation, it is still difficult to study this nanoscale protein organization. To tackle this challenge, we have developed a new branched proximity hybridization assay (bPHA). In this assay, target proteins are first bound by specific antibodies, antibody fragments (Fab, scFv, VHH) or aptamers coupled to specially designed (plus and minus) oligonucleotides. A nanoscale distance between two target proteins places the plus and the minus oligonucleotide in close vicinity. This close vicinity signal is then amplified by 400 times through a sequential hybridization processes using the branched DNA (bDNA) technique. With the new bPHA method the nanoscale protein-protein interactions on or inside cells can be measured by flow cytometry in a multiplexing, high-throughput and quantitative manner. Deploying bPHA, we reliably detected the intermixing of B cell receptor isotypes upon B cell stimulation. We were also able to measure the intracellular dynamics of Syk kinase recruitment to the BCR signaling subunit after treatment of B cells with different stimuli.

WS.A4.01.03

Reconstitution of T-cell synaptic ectosomes unravels vesicular CD40L density as a critical determinant in the feed forward activation of antigen presenting cells

***P. F. Céspedes-Donoso**[1], D. G. Saliba[1], S. Valvo[1], S. Balint[1], K. Korobchevskaya[1], E. Compeer[1], M. Tognolli[2], E. O´Neill[2], M. L. Dustin[1];*
[1]The Kennedy Institute of Rheumatology, University of Oxford, Oxford, United Kingdom, [2]Department of Oncology, University of Oxford, Oxford, United Kingdom.

Extracellular vesicles are important intercellular communication elements across tissues. Recently, we have described synaptic ectosomes (SE) as a specialized type of extracellular vesicle that both form in response to antigen receptor stimulation and are released into the synaptic cleft by T-cells. However, the nature and functionality of the protein cargo remain largely unknown. Here, we developed a bead supported lipid bilayer system (BSLB) linked to quantitative flow cytometry (FCM) for characterizing SE composition and test their functionality using synthetic small unilamellar vesicles (SUVs). CD4+ T-cells were stimulated in the presence of BSLBs containing ICAM-1/anti-CD3-Fab/+/- CD40/ICOSL. After dissociation of BSLB-T-cell conjugates, BSLBs and cells were analyzed by FCM to characterize the transfer dynamics of CD40L molecules, a key effect of helper T-cells. To assess functionality, we developed SUV that both have the same size, and contained equivalent densities of CD40L as SE. We observed that SE were enriched in CD40L when the bilayers contained anti-CD3-Fab and CD40. Interestingly, the synaptic transfer of CD40L was triggered at low anti-CD3 densities and then plateaued at higher anti-CD3, unlike TCR transfer, which was linear with anti-CD3 density. The density of CD40L on SE was 100-1000 fold higher than the average calculated density on the surface of T-cells. CD40L on SE appears to be the dominant form leading the feed-forward activation of APCs, as evidenced by a superior APC activation compared to equivalent soluble CD40L. Funding: ERC AdG 670930, Wellcome Trust 100262, Kennedy Trust, NIH AI043542, NIH tetramer core facility, EMBO ALTF 1420-2015.

WS.A4.01.04

In follicular regulatory T cells NFATc1 is essential for homing to germinal centers

***A. Koenig**[1], M. Vaeth[1], G. Müller[2], S. Klein-Hessling[1], M. Klein[3], D. Stauss[2], L. Dietz[1], T. Bopp[3], M. Lipp[2], I. Berberich[4], F. Berberich-Siebelt[1];*
[1]Institute of Pathology, Julius-Maximilians-University of Wuerzburg, 97080 Würzburg, Germany, [2]Department of Tumor Genetics and Immunogenetics, Max-Delbruck-Center for Molecular Medicine (MDC), 13092 Berlin, Germany, [3]Institute for Immunology, University Medical Center, University of Mainz, 55131 Mainz, Germany, [4]Institute for Virology and Immunobiology, University of Wuerzburg, 97080 Würzburg, Germany.

Plasma cells, secreting class-switched antibodies with high affinity, are the product of the germinal center reaction (GCR). The GCR is supported by CD4$^+$CXCR5$^+$ follicular T-helper cells (T_{FH}), and controlled by CD4$^+$CXCR5$^+$Foxp3$^+$ follicular regulatory T-cells (T_{FR}). Expression profiling revealed high expression of the 'Nuclear Factor of Activated T cells c1' (NFATc1) in follicular T cells, covering T_{FH} as well as T_{FR} cells. The ablation of *Nfatc1* in all T cells, but also in Foxp3-expressing cells only, led to an increase in the GCR. This effect was due to an impaired homing of the T_{FR} population to the B-cell follicle, because T_{FR} cells specifically failed to upregulate the homing receptor CXCR5.

In T_{FR} cells - in contrast to T_{FH} cells - 'B lymphocyte-induced maturation protein' (Blimp-1) is highly expressed in line with being a hallmark of effector regulatory T-cells. Our data indicated that Blimp-1 directly represses the expression of *Cxcr5* in Tregs. However, it supports the recruitment of NFATc1 to *Cxcr5* by protein-protein interaction, by those means cooperating with NFATc1 for transactivation of *Cxcr5*. In line, overexpressing constitutive active NFATc1 in post-thymic T cells supported CXCR5 expression on Tregs. Interestingly, the numbers in T_{FH} and germinal center B cells were so deeply reduced that a surplus in NFATc1 might even enhance the effector function of T_{FR} cells. In sum, NFATc1 is essential for overcoming Blimp-1-mediated repression of *Cxcr5* and therefore for homing of T_{FR} cells, which control the GCR, an essential part of the humoral immune response.

WS.A4.01.05

Ectopic lung resident germinal centres are formed during chronic house dust mite driven allergic airway disease in a T follicular helper cell dependent manner

***F. I. Uwadiae**, R. Siroya, A. Gerasimov, C. Spilsbury, C. J. Pyle, S. A. Walker, C. M. Lloyd, J. A. Harker;*
Imperial College London, London, United Kingdom.

Introduction: Allergic asthma is a disease of chronic allergen exposure, characterised by airway inflammation, airway hyperresponsiveness and allergen specific IgE. Germinal centres (GC) are anatomically distinct structures, located primarily in secondary lymphoid organs, critical for antibody generation. Lymphocyte aggregates exist in the asthmatic lung, but their formation and contribution to disease is not understood. This study aims to understand ectopic GCs during chronic allergic airway disease (AAD).
Method: To establish chronic AAD, mice were repeatedly exposed to intranasal house dust mite for up to 5 weeks.
Results: GC B cells and T follicular helper cells (Tfh) were found in the mediastinal lymph nodes (mLN) and the lungs after 3 weeks of allergen exposure. Large B cell aggregates containing GC B cells, T cells and follicular dendritic cell (FDC) networks were identified in the lungs by confocal microscopy, indicative of an active GC. Sorting mLN and lung resident B cells revealed transcripts indicative of IgG1 and IgE switch in both compartments by qPCR, while IgA transcripts were only identified in the lungs, suggesting the lungs to also provide protective antibodies. Ectopic GCs were absent in Tfh deficient mice (*$Cd4^{cre}Bcl\text{-}6^{fl/fl}$*), which additionally lacked allergen specific IgE. Despite this, *$Cd4^{cre}Bcl\text{-}6^{fl/fl}$* mice had exaggerated Th2 cell biased AAD. Mixed bone marrow chimeras revealed a regulatory role for Tfh during AAD, rather than an intrinsic role for Bcl6 in regulating Th2 cell differentiation. **Conclusions:** Lung resident GCs form during chronic AAD in a Tfh dependent manner and Tfh are important regulators of disease.

WS.A4.01.06

Antigen stimulation of lymphoid cells supports alternative pathway of T Follicular helper cell differentiation by polarizing CD45RA$^-$ CD4 T cells into Tfh cells

***R. Jeger-Madiot**[1], M. Pereira[1], C. Richetta[2], V. Quiniou[3], P. Buffet[4], D. Klatzmann[3], A. Moris[1], S. Graff-Dubois[1];*
[1]Centre d'Immunologie et des Maladies Infectieuses, Paris, France, [2]Ecole Normale Supérieure Paris Saclay, Paris, France, [3]Laboratoire Immunologie-Immunopathologie-Immunothérapie, Paris, France, [4]Institut National de la Transfusion Sanguine, Paris, France.

Lymphoid environment and antigenic stimulation synergize to generate Tfh cells that promote B cell maturation. In chronic infections, Tfh cell frequency is increased. Chronic antigen stimulation might promote Tfh cell differentiation leading to pathogenic antibodies. Understanding the pathways of Tfh differentiation and GC reaction under pathologic conditions is of particular interest to develop new therapeutic approaches. Tfh cells are not easily accessible, limiting their study in humans. Here, we provide a culture system to generate fully differentiated Tfh cells, allowing to understanding the Tfh biology. Our culture system use human splenocytes stimulated with a superantigen and suitable cytokines. We showed that induced Tfh cells present hallmarks of autologous Tfh cells, expressing Tfh markers and promoting B cell maturation. Compared with PBMC, Tfh cell differentiation is much more efficient with splenocytes in terms of quantity. This suggests that lymphoid environment promotes Tfh differentiation. Using this culture system, we demonstrated that Tfh could differentiate from CD45RA$^-$ CD4 T cells and naive T cells. We are currently performing experiments to characterize the functional properties of Tfh cells generated from naïve and repolarized CD4$^+$ T cells. To our knowledge, this is the first evidence of a CD4 T cell repolarization into Tfh cells in humans. CD4$^+$ T cell repolarization into Tfh cells might contribute to increase Tfh cell frequency during chronic inflammatory diseases where lymphoid structures are ectopically induced. Using this culture system will provide insights on Tfh cell differentiation/functions. Looking ahead, this culture system will constitute an platform to test new therapeutic approaches.

WS.A4.02 Regulation of B cell development and differentiation

WS.A4.02.01

B cell positive selection is developmentally regulated during ontogeny by the heterochronic protein Lin28b

***S. Vanhee**, S. Datta, T. Kristiansen, H. Åkerstrand, S. Lang, S. Vergani, A. Doyle, K. Olsson, S. Soneji, E. Jaensson Gyllenbäck, J. Yuan;*
Department of Molecular Hematology, Biomedical Center (BMC), Lund University, Lund, Sweden.

While all T cell maturation requires self-antigen driven positive selection, most self-reactive B cells are subject to tolerance induction mechanisms. An exception to the rule is the CD5$^+$ B-1 cell population known to be primarily of fetal and neonatal origin, generated and maintained on the basis of their self-reactivity. However, the mechanisms underlying their positive selection during early life and their subsequent developmental attenuation remains unclear.
Here, we link surface CD5 levels to BCR self-reactivity within the B-1 compartment and show that CD5 is induced at the immature B cell stage during neonatal but not adult B cell maturation coinciding with B cell positive selection. Importantly, developmental induction of CD5 relies in a dose dependent manner on the heterochronic RNA-binding protein Lin28b. Ectopic Lin28b effectively reinitiates positive selection during adult B cell maturation and increases the progeny:precursor ratio of B cell selection by two fold as shown by cellular barcoding. Finally, our results uncouple the process of B cell positive selection from the semi-invariant repertoire and phosphatidylcholine reactivity characteristic for B-1 cells. Together, our data support a model in which developmentally restricted Lin28b expression potentiates a transient wave of B cell positive selection and thereby contributes to life-long heterogeneity within the B cell pool.

WS.A4.02.02

The bHLH transcription factor TCFL5 and its soform CHA differently modulate c-MYC-dependent B cell proliferation

***I. Sánchez-Gómez**, J. Galán-Martínez, M. Maza, K. Stamatakis, N. Gironès, M. Fresno;*
Centro de Biología Molecular Severo Ochoa, Madrid, Spain.

Modern lifestyle and increased average lifespan are causing a world-wide increase in cancer rates. c-MYC basic Helix-Loop-Helix (bHLH) transcription factor is key in the genesis of several types of cancer. Deregulation of c-MYC has been frequently associated with aggressive lymphomas and adverse clinical outcome in B-cell malignancies. Trancription factor TCFL5 and its isoform CHA are also members of bHLH family. Since c-MYC and TCFL5 belong to the bHLH family they may be able to form heterodimers, thus the aim of this study is to analyze whether TCFL5/CHA interact with c-MYC and if it also regulates its functionality in a cancer environment. Our results showed a direct interaction of c-MYC and CHA by co-immunoprecipitation assays. Then, we performed gene expression studies to analyze the effect that the overexpression of CHA in presence or absence of c-MYC and found inhibition c-MYC target genes expression by CHA. In addition, TCFL5/CHA stably silenced cell lines were generated that confirmed our hypothesis about the modulatory role of TCFL5 and CHA in c-MYC mediated responses. Finally, protein expression analysis in acute lymphoblastic leukemia-like activated B cells showed differential effects of TCFL5 and CHA in cell proliferation. In summary, we found that CHA downregulates c-MYC activity and as a result it reduced cell tumorogenicity. On the contrary, TCFL5 expression was induced during B cell activation and was related to an immature stage contributing to a tumorigenic phenotype. Further studies may unravel CHA as a possible therapeutic target not only in cancer but also in leukemia.

WS.A4.02.03

PI3Kδ hyper-activation in B cells promote increased susceptibility to *S. pneumoniae* airway infection through an antibody independent mechanism

***A. Stark**[1,2], A. Chandra[1,2], R. Alam[2], K. Okkenhaug[1,2];*
[1]Division of Immunology, Department of Pathology, University of Cambridge, CB2 1QP, Cambridge, United Kingdom, [2]Babraham Institute, Cambridge, CB22 3AT, UK, Cambridge, United Kingdom.

The PI3Kδ signalling pathway is critical for normal immune cell development and function. Gain of function mutations affecting the p110δ catalytic- or p85α regulatory subunits causes Activated PI3K-delta Syndrome (APDS), a primary immunodeficiency characterised by severe recurrent respiratory infections often caused by *S. pneumoniae*.
We generated conditional knockin mouse models of PI3Kδ hyper-activation (PI3Kδ^{E1020K}) and PI3Kδ inactivation (PI3Kδ^{D910A}) to study the role of PI3Kδ signalling in the immune response to respiratory infection.
Germline and B cell, but not T cell or myeloid, restricted PI3Kδ hyper-activation increases susceptibility to *S. pneumoniae* lung infection in PI3Kδ^{E1020K} mice. Kinase-inactive PI3Kδ^{D910A} mice were not more susceptible to infection, despite lacking natural antibody against *S. pneumoniae*. Furthermore, PI3Kδ hyper-activation does not limit natural antibody levels or an antibody response to Pneumovax, a T-independent vaccine. Mice lacking mature B cells (μMT) are also protected against acute disease but fail to clear the infection, highlighting the pathological role of B cells in this model.
These data indicate that, while antibodies are important in the immune response to *S. pneumoniae*, B cells can play an antibody independent detrimental role during acute lung infection, and this effect is exacerbated by PI3Kδ hyper-activation. Indeed, we found an atypical IL-10 producing CD19$^+$B220$^-$ B cell subset which is significantly expanded in PI3Kδ^{E1020K} mice and absent in PI3Kδ^{D910A} mice. Ongoing work focusses on elucidating the mechanism whereby these cells can contribute to pathology in the early phase of *S. pneumoniae* infection.
This work is funded by grants from the MRC and Wellcome Trust.

WS.A4.02.04

Expansion of activated CXCR5$^+$ICOS$^+$Tfh cells and plasmablasts induced by seasonal influenza vaccine is impaired in anti-IL-6R treated rheumatoid arthritis patients

*A. Agua-Doce[1], V. Romao[2], J. Polido-Pereira[2], R. Barros[3], I. P. Lopes[1], M. I. Seixas[3], M. J. Saavedra[3], E. Sacadura-Leite[4], H. Rebelo-de-Andrade[5], J. E. Fonseca[2], **L. Graca**[1];*
[1]Instituto de Medicina Molecular, Lisboa, Portugal, [2]Instituto de Medicina Molecular, Rheumatology Department, Hospital Santa Maria-CHLN, Lisboa, Portugal, [3]Rheumatology Department, Hospital Santa Maria-CHLN, Lisboa, Portugal, [4]Occupational Medicine Department, Hospital Santa Maria-CHLN, Lisboa, Portugal, [5]Instituto Nacional de Saúde Doutor Ricardo Jorge, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal.

Identification of a T cell subset (Tfh) with access to B cell areas and in driving the GC reaction has shed light to the unique processes of development and control of these structures. Tfh cells are essential for the generation of high affinity neutralizing antibodies elicited following vaccination, and are also involved in immune dysregulation associated with immune-mediated inflammatory diseases (IMID) and, specifically, to ectopic GC formation. Murine studies have shown that interleukin (IL)-6 is required for Tfh differentiation. However, in humans this issue has not been addressed *in vivo*. Our study takes advantage from the fact that tocilizumab (TCZ), an IL-6R-blocking monoclonal antibody (MAb), is approved for RA treatment of patients with indication for influenza vaccination. We studied prospectively a cohort of RA patients treated with TCZ (n=42) or methotrexate (MTX, n=42), as well as healthy volunteers (HD, n=53), following influenza vaccination. We found marked expansion of activated CXCR5$^+$ICOS$^+$ Tfh population at day 7 post vaccination in HD and MTX-treated patients, but this was impaired in the TCZ group. The increase in the activated CXCR5$^+$ICOS$^+$ Tfh population was mainly due to the Th1-like (CXCR3+) Tfh subpopulation. The lack of expansion of CXCR5$^+$ICOS$^+$ Tfh cells in TCZ-treated patients was due to inhibition of their proliferative ability, as demonstrated by reduced Ki67$^+$CD38$^+$. Taken together we found *in vivo* IL-6 blockade in humans hampers the emergence of activated CXCR5+ICOS+ Tfh cells following influenza vaccination, suggesting IL-6 has an important role in the generation of activated Tfh cells and protective humoral immune responses.

WS.A4.02.05

Functions of ZBTB24 in human and murine B cells

*C. Zhu[1], Y. Zhao[2], J. Liang[1], X. Gao[1], **J. Wang**[1];*
[1]Institutes of Biology and Medical Sciences, Suzhou, China, [2]Department of Pathophysiology, School of Biology and Basic Medical Sciences, Suzhou, China.

ZBTB24 has been recently identified as the causative gene in patients with Immunodeficiency, Centromeric Instability and Facial Anomalies syndrome type 2 (ICF2), a rare autosomal recessive disease. Most ICF2 patients harbor *ZBTB24* nonsense mutations, and suffer from recurrent respiratory and gastrointestinal infections due to hypogammaglobulinemia, most likely due to the lack of germinal center (GC) structure and circulating CD19$^+$CD27$^+$ memory B cells (Bmem). ZBTB24 belongs to the large ZBTB family of transcriptional repressors with members like BCL6 (ZBTB27) & ZBTB7A (LRF) playing essential roles in B-cell development/functions. ZBTB24 is highly expressed in human B-cell compartment. Knockdown of endogenous ZBTB24 hampers the cell-cycle progression in human GC-derived B lymphoma Raji cells *via* upregulating the expressions of IRF4 & PRDM1, two essential transcriptional factors invovled in GC-reactions. Moreover, ZBTB24 exerts these functions independent of BCL6 as it neither heterodimerizes with nor affects the expression/transcriptional activity of BCL6. Collectively, ZBTB24 appears to control the *in vivo* Bmem development *via* regulating the proliferation and/or terminal differentiation of human GC-B cells. Intriguingly, despite the early embryonic lethality in conventional zbtb24-deficient mice, conditional-knockout of *zbtb24* in murine B cells has no significant impact on *in-vivo* antibody responses upon immunization with T-cell-dependent antigens. Thus, functions of ZBTB24 in B cells seem to differ in mice and humans.
Funding acknowledgements: This study was financially supported by the National Key Research and Development Program of China (2017YFB0403805); National Natural Science Foundation of China (31670888/31370871/81470564); Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) and Ministry of Education (IRT1075).

WS.A4.02.06

Stem cell based gene therapy for Recombinase deficient-SCID

***F. J. T. Staal**, K. Pike-Overzet;*
LUMC, Leiden, Netherlands.

Recombinase-activating gene (RAG) deficient SCID patients lack B and T lymphocytes due to the inability to rearrange immunoglobulin and T-cell receptor genes. The two *RAG* genes are acting as a required dimer to initiate gene recombination. Gene therapy is a valid treatment alternative for RAG-SCID patients, who lack a suitable bone marrow donor, but developing such therapy for RAG1/2 has proven challenging. Hence, we tested clinically relevant lentiviral SIN vectors with different internal promoters (UCOE, PGK, MND, and UCOE-MND) driving codon optimized versions of the *RAG1* or *RAG2* genes to ensure optimal expression. We used *Rag1-/-* or Rag2-/-mice as a preclinical model for RAG-SCID to assess the efficacy of the various vectors at low vector copy number. In parallel, the-conditioning regimen in these mice was optimized using busulfan instead of commonly used total body irridiation. We observed that B and T cell reconstitution directly correlated with RAG1 and RAG2 expression. Mice receiving low Rag1/2 expression showed poor immune reconstitution; however high Rag1/2 expression resulted in a lymphocyte reconsitution comparable to mice receiving wild type stem cells. Efficiency and safety of our clinical RAG1 lentivirus batch was assessed in Rag1-/- mice model showing that functional restoration of RAG1-deficiency can be achieved with clinically acceptable vectors. Additionally, RAG1-SCID patient CD34$^+$ cells transduced with our clinical RAG1 vector and transplanted into NSG mice led to fully restored human B and T cell development. Together with favourable safety data, these results substantiate a clinical trial for RAG1 SCID which is planned for late 2018.

WS.A5.01 Innate effectors in the onset of immune responses

WS.A5.01.01

Inflammation-induced cancer and cancer-induced inflammation in colon: a role for S1P lyase

***A. Schwiebs**[1], M. Herrero[1], E. Wiercinska[2], M. Anlauf[3], F. Ottenlinger[1], K. Schmidt[1], D. Thomas[1], E. Elwakeel[1], A. Weigert[1], H. Bonig[2], K. Scholich[1], G. Geisslinger[1], J. M. Pfeilschifter[1], H. H. Radeke[1];*
[1]Clinic of the Goethe University Frankfurt, Frankfurt, Germany, [2]German Red Cross Blood Service Institution, Frankfurt, Germany, [3]St. Vincenz Hospital, Limburg, Germany.

Objective: In this study we investigated the compartment-specific sphingolipid modulation for the development of colitis-associated colorectal cancer (CAC) and found that sphingolipids can account for both mechanisms: inflammation-induced cancer and cancer-induced inflammation. **Design:** We performed isogenic bone marrow transplantation of inducible Sphingosine-1-phosphate (S1P) lyase knockdown mice to specifically modulate sphingolipids and associated genes and proteins in a compartment-specific way in a DSS/AOM mediated CAC model. 3-D organoid cultures were used *in vitro*. **Results:** In our CAC model, S1P lyase (SGPL1) knockdown in either tissue cells or immune cells, caused compartment-specific actions resulting in a morphologically, genetically and time-dependently distinct development of inflammation and carcinogenesis in colon: Immune cell SGPL1 knockdown (I-SGPL$^{-/-}$) augmented severe leukocyte and moreover lymphocyte infiltration of a mixed T_h1 and T_h2 phenotype, initiating strong colitis with lesions. Subsequent pathological crypt remodeling and extracellular S1P-signaling facilitated emergence of tumors characterized by PDCD4 regulation. In contrast, tissue SGPL1 knockdown (T-SGPL$^{-/-}$) provoked immediate occurrence of epithelial-driven tumors. Carcinogenesis was accompanied by an IL-12/IL-23 shift and sphingosine kinase 1, S1P receptor 2 and epidermal growth factor receptor upregulation with a consecutive development of a T_h2-driven microenvironment. Moreover, both knockdown models showed distinct regulation of lymphopenia und neutrophilia, different from the global SGPL knockout. **Conclusion:** Our results demonstrate that cell type-specific sphingolipid modulation contributes to the development of either inflammation-induced cancer or cancer-induced inflammation.

WS.A5.01.02

CD28 co-stimulation during antigenic recall responses of CD4$^+$Th1 cells triggers IFNg secretion in vivo

***S. Haack**[1], D. Langenhorst[1], F. Lühder[2], T. Hünig[1], N. Beyersdorf[1];*
[1]Institute for Virology and Immunobiology, Würzburg, Germany, [2]Institute for Multiple Sclerosis Research and Neuroimmunology, Göttingen, Germany.

During differentiation of naïve CD4$^+$T cells CD28-mediated co-stimulation is known to play a crucial role determining the cells' fate. The relevance of CD28 costimulatory signals for antigenic recall of CD4$^+$T cell is, however, less clear. Therefore, we first differentiated T cells from OT-II TCR-transgenic mice *in vitro* into Th1 cells and then transferred them into antigen-naive recipient mice *in vivo*. Three days after T cell transfer we challenged the recipients with OVA antigen in alum. CD28 signaling was abrogated either by Tamoxifen treatment of recipient mice after using OT-II T cells from inducible CD28 KO mice or by application of Fab fragments of the αCD28 mAb E18 inhibiting ligand binding to CD28. As read-outs we measured cytokine concentrations in the serum 6, 24 and 48 h after antigenic challenge and re-isolated the OT-II T cells ten days after transfer to determine absolute cell numbers and to detect IFNγ, IL-4, T-bet and Gata-3 expression by intracellular staining. Our results show that neither expansion nor maintenance of the Th1 phenotype was influenced by CD28-mediated co-stimulation *in vivo*. In contrast, systemic IFNγ release was greatly enhanced upon CD28 co-stimulation of the donor OT-II T cells. Inhibiting CD28-triggered cytokine release from effector/memory Th1 cells may, thus, be used in treatment of autoimmune diseases like multiple sclerosis. This study was funded by grants from the DFG (BE4080/2-1 & Hu295/12-1) and the GSLS Würzburg (GSC106/3).

WS.A5.01.03

Blood transcriptomic analysis shows how recall innate responses are modulated by the use of an adjuvant at priming

E. Pettini[1], *F. Santoro*[1], *D. Kazmin*[2], *A. Ciabattini*[1], *F. Fiorino*[1], *G. Gilfillan*[3], *I. Evenroed*[3], *P. Andersen*[4], *G. Pozzi*[1], *D. Medaglini*[1];
[1]*Laboratorio di Microbiologia Molecolare e Biotecnologia (LA.M.M.B.), Siena, Italy, Italy,* [2]*Emory Vaccine Center, Emory University,, Atlanta, GA, United States,* [3]*Department of Medical Genetics, Oslo University Hospital and University of Oslo, Oslo, Norway,* [4]*Department of Infectious Disease Immunology, Statens Serum Institut,, Copenhagen, Denmark.*

Transcriptomic profiling of the immune response induced by vaccine adjuvants is of critical importance for the rational design of vaccination strategies. In the present study, we investigated how the vaccine adjuvant used for priming modifies the way the immune system responds to the re-exposure to the vaccine antigen alone. mRNA sequencing was performed on blood samples collected after priming and boosting from mice primed with the vaccine antigen H56 of *Mycobacterium tuberculosis* administered alone or with the CAF01 adjuvant and boosted with the antigen alone. Gene expression analysis 2 days after priming showed that the CAF01 adjuvanted vaccine induced a stronger upregulation of the innate immunity modules compared to the unadjuvanted formulation. The immunostimulant effect of CAF01 adjuvant, used for priming, was clearly seen also one day after boosting, with activation of blood transcription modules related to innate immune response, such as monocyte and neutrophil recruitment, activation of antigen presenting cells and interferon response. The analysis of the immune response showed a higher frequency of H56-specific CD4+ T cells and germinal center B cells in draining lymph nodes and a strong H56-specific humoral response in mice primed with H56 + CAF01. Transcriptomic analysis of isolated H56-specific $CD4^+$ T cells was also conducted to profile gene expression in the mature antigen-specific helper T cell population upon vaccination. These data indicate that the adjuvant used for priming strongly re-programs the immune response that, upon boosting, results in a stronger recall of the innate response essential for shaping the downstream adaptive response.

WS.A5.01.04

DNGR-1 dampens neutrophil recruitment to damaged tissues, fostering disease tolerance upon infection

C. DEL FRESNO SÁNCHEZ[1], *P. Saz-Leal*[1], *M. Enamorado*[1], *S. Wculek*[1], *S. Martínez-Cano*[1], *N. Blanco-Menéndez*[1], *O. Schulz*[2], *M. Gallizioli*[3], *F. Miró*[3], *E. Cano*[4], *A. Planas*[3], *C. Reis e Sousa*[2], *D. Sancho*[1];
[1]*FUNDACION CNIC, MADRID, Spain,* [2]*The Francis Crick Institute, London, United Kingdom,* [3]*Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain,* [4]*Unidad Funcional De Investigación De Enfermedades Crónicas, Instituto De Salud Carlos III, Majadahonda, MADRID, Spain.*

Introduction: DNGR-1-(*Clec9a*) is a dead-cell receptor mainly expressed on type-I dendritic cells (DC1s) implicated in cross-presentation of dead cell-associated antigens to $CD8^+$ T-cells. We propose that DNGR-1 also impacts on innate immune responses.
Methods: WT mice and DNGR-1-deficient were subjected to sterile (acute necrotizing pancreatitis) and infectious (systemic *Candida albicans* infection) tissue damage models, analyzing myeloid infiltration and the tissue injury extent. Same models were addressed in anti-DNGR-1-treated WT mice or DNGR-1-deficient mice lacking adaptive immunity. To address the role of neutrophils to the magnitude of the observed pathology, mice were treated with neutrophil-depleting antibodies. The inflammatory response of DC1s to some PAMPs was analyzed after *in vitro* exposition to a ligand for DNGR-1. The implication of SHP-1 was addressed *in vitro* through a chemical inhibitor. The expression of the neutrophil chemoattractant Mip-2/CXCL2 was analyzed in sorted haematopoietic populations from *C.albicans*-infected kidneys.
Results: DNGR-1 absence or blockade led to exacerbated caerulein-induced pancreatitis. Similarly, DNGR-1-deficient settings increased pathology during *Candida* infection without affecting fungal burden, suggestive of a disease tolerance-related process. Both exacerbated responses were independent of adaptive immunity and attributable to increased neutrophilia. Ligand engagement by DNGR-1 activates SHP-1 to dampen inflammatory responses, decreasing the expression of the neutrophil chemoattractant Mip-2/CXCL2. Among the renal immune infiltrate of *Candida*-infected DNGR-1-deficient mice, DC1s were the only population overexpressing Mip-2/CXCL2.
Conclusions: Tissue damage sensing by DNGR-1 in DC1s negatively regulates Mip-2/CXCL2 expression, reducing host-damaging neutrophil infiltration. Upon infectious conditions, this immunomodulation occurs without affecting pathogen burden, suggesting that DNGR-1 could promote disease tolerance.

WS.A5.01.05

Toll-like receptor signalling induces a temporal switch from inflammatory towards a more resolving lipid profile in monocyte-derived macrophages

J. von Hegedus[1], *M. Heijink*[2], *T. Huizinga*[1], *M. Kloppenburg*[1,3], *M. Giera*[2], *R. Toes*[1], *A. Ioan-Facsinay*[1];
[1]*Department of Rheumatology, 2333 ZA, Netherlands,* [2]*Center for Proteomics and Metabolomics, 2333 ZA, Netherlands,* [3]*Department of Clinical Epidemiology, Leiden, Netherlands.*

Background: Inflammation is a tightly regulated process that usually resolves spontaneously. Dysregulation of this process can lead to chronic inflammation. Several cells and soluble mediators, including lipid mediators, regulate the course of inflammation and its resolution. Previous data suggest a temporal lipid-mediator switch from pro-inflammatory lipid mediators at the start of inflammation towards specialized pro-resolving lipid mediators (SPM) during the resolution phase of inflammation. It is, however, unclear which signals initiate secretion of SPM and the resolution process. Macrophages are key players in regulating tissue inflammation through secretion of soluble mediators , including lipid mediators.
We hypothesize that the initiation of resolution is orchestrated by macrophages in response to persistent inflammatory stimuli.
Methods: M1 polarized monocyte-derived macrophages were stimulated with LPS for different periods of time. Changes in lipid profile were measured using liquid chromatography coupled to tandem mass spectrometry. In parallel, expression of cyclooxygenase and lipoxygenases-15 was determined using qPCR. Additionally, lL-10 and TNFa ELISA's were performed on monocyte-derived macrophages that were pre-incubated with lipid mediators.
Results: Twenty-four different lipids were detected in LPS-stimulated macrophages. Cyclooxygenase-derived pro-inflammatory prostaglandins were observed in the first six hours of stimulation. Interestingly, a switch towards the 15-lipoxygenase SPM precursors 15-HETE and 17-HDHA was observed after 24h. The mRNA expression of cyclooxygenase and 15-lipoxygenase was in line with this trend. Treatment of macrophages with 17-HDHA during stimulation decreased TNFα and increased IL-10 production, indicating the anti-inflammatory properties of 17-HDHA.
Conclusion: Macrophages can initiate the resolution of inflammation in response to persistent inflammatory stimuli.

WS.A5.01.06

OX40 is required for both Th17 and Th1 effector T cell programmes

D. Gajdasik[1], *C. Willis*[1], *R. Fiancette*[1], *M. Botto*[2], *T. Vyse*[3], *G. Frankel*[2], *D. Ruano-Gallego*[2], *D. Withers*[1];
[1]*University of Birmingham, Birmingham, United Kingdom,* [2]*Imperial College London, London, United Kingdom,* [3]*King's College London, London, United Kingdom.*

Whilst it is clear that CD4 T cell responses require signals through OX40 and CD30, the cellular sources of the ligands and exactly what benefits they confer to the response have not been fully elucidated.
Our unpublished studies using an attenuated *Listeria monocytogenes*-2W1S infection model have demonstrated that IFNg production by effector T cells is highly OX40L dependent. Furthermore CD11c+ DCs are the critical cellular source of OX40L during this primary response, with OX40L expression by T cells and ILC3 redundant.
To test whether the Th17 effector response shares the OX40 requirements identified in our systemic Th1 model, we have analysed the role of OX40 in intestinal Th17 responses. Mice deficient in OX40 and CD30 show a dramatic defect in intestinal IL-17A-producing CD4 T cells upon *ex vivo* restimulation and RORgt-expressing CD4 T cells are absent from this tissue.
To further dissect the requirement for OX40 and CD30 in Th17 responses, Smart17 (IL-17A) reporters and Smart17 x OX40-/- x CD30-/- mice were infected with a novel *Citrobacter rodentium*-2W1S strain and the antigen specific response assessed. 2W1S-specific effector CD4 T cells in the intestine were substantially reduced and Citrobacter fecal counts significantly elevated. Thus, the Th17 effector programme in the intestinal lamina propria appears to also be highly OX40L dependent and ongoing experiments with conditional knockout mice are dissecting whether DC or ILC3 are key sources of OX40L.
Combined these data demonstrate the fundamental role played by OX40 in establishing functional effector CD4 T cells in both Th1 and Th17 responses.

WS.A5.02 Early T cell functions in immune responses

WS.A5.02.01

Mature CD10+and immature CD10-neutrophils present in G-CSF-treated donors display opposite effects on T cells

O. Marini[1], *S. Costa*[1], *D. Bevilacqua*[1], *F. Calzetti*[1], *N. Tamassia*[1], *C. Spina*[2], *D. De Sabata*[2], *E. Tinazzi*[3], *C. Lunardi*[3], *M. T. Scupoli*[4], *C. Cavallini*[4], *E. Zoratti*[5], *I. Tinazzi*[6], *A. Marchetta*[7], *A. Vassanelli*[8], *M. Cantini*[8], *G. Gandini*[8], *A. Ruzzenente*[9], *A. Guglielmi*[9], *F. Missale*[10], *W. Vermi*[10], *C. Tecchio*[2], *M. A. Cassatella*[1], *P. Scapini*[1];
[1]*Department of Medicine, Division of General Pathology, University of Verona, Verona, Italy,* [2]*Department of Medicine, Division of Hemathology, University of Verona, Verona, Italy,* [3]*Department of Medicine, Division of Internal Medicine, University of Verona, Verona, Italy,* [4]*Interdepartmental Laboratory of Medical Research, University of Verona, Verona, Italy,* [5]*Interdepartmental Laboratory of Medical Research, Applied Research on Cancer-Network, University of Verona, Verona, Italy,* [6]*Rheumatology Unit, Division of General Medicine, Sacro Cuore Hospital of Negrar, Verona, Italy,* [7]*Rheumatology Unit, Division of General Medicine, Sacro Cuore Hospital of Negrar, Verona, Italy, Verona, Italy,* [8]*Transfusion Medicine Department, Integrated University Hospital, Verona, Italy,* [9]*Department of Surgery, Division of General Surgery "A," University of Verona, Verona, Italy,* [10]*Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy.*

The identification of neutrophil populations, as well as the characterization of their immunoregulatory properties, is an emerging topic under extensive investigation. In such regard, the presence of circulating CD66b$^+$ neutrophil populations, exerting either immunosuppressive or proinflammatory functions, has been described in several acute and chronic inflammatory conditions. However, due to the lack of specific markers, the precise phenotype and maturation status of these neutrophils remain unclear. Herein, we report that CD10 can be used as a marker that, within heterogeneous populations of circulating CD66b$^+$ neutrophils present in inflammatory conditions, clearly distinguishes the mature from the immature ones. Accordingly, we observed that the previously described immunosuppressive neutrophil population that appears in the circulation of granulocyte colony-stimulating factor (G-CSF)–treated donors (GDs) consists of mature CD66b$^+$ CD10$^+$ neutrophils displaying an activated phenotype. These neutrophils inhibit T cell functions via a CD18-mediated contact-dependent arginase 1 release. By contrast, we found that immature CD66b$^+$ CD10$^-$ neutrophils, also present in GDs, display an immature morphology, promote T-cell survival and functionality. Altogether, our findings uncover that in GDs, circulating mature and immature neutrophils exert opposite immunoregulatory properties. Therefore, CD10 might be used as a phenotypic marker discriminating neutrophil populations present in patients with acute or chronic inflammatory conditions, as well as facilitating their isolation, to better define their specific immunoregulatory properties. (Blood. 2017;129(10):1343-1356)

WS.A5.02.02

Tight translational control of glycolysis and fatty acid metabolism regulates the transition of CD4$^+$ T cells from quiescence to metabolic remodelling

S. Ricciardi[1], *N. Manfrini*[1], *R. Alfieri*[1], *P. Calamita*[1], *M. Crosti*[1], *R. Muller*[2], *M. Pagani*[1,3], *S. Abrignani*[1,4], *S. Biffo*[1,5];
[1]*National Institute of Molecular Genetics, Milan, Italy,* [2]*Helmholtz Institute for Pharmaceutical Research, Saarbrücken, Germany,* [3]*Department of Medical Biotechnology and Translational Medicine, Universita' degli Studi di Milano, Milan, Italy,* [4]*Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy,* [5]*Bioscience Department, Università degli Studi di Milano, Milan, Italy.*

Upon antigen encounter via TcR, naïve cells undergo a metabolic reprogramming, which supports growth and imprints distinct functional fate, but the molecular basis for this is unclear. Integrating multiple "omics" analysis of human resting and naïve cells following activation, we discovered that T cells exert the transitional process through translational control. Naïve cells are poised at the preinitiation step of translation, accumulate untranslated mRNAs encoding for glycolysis and fatty acid synthesis factors, and present a unique metabolomic profile. Upon TcR engagement, activation of the translational machinery leads to synthesis of GLUT1 protein that steers glucose entry. Next, translation of ACC1 mRNA, via eIF4E, completes metabolic reprogramming toward an effector phenotype. Notably, inhibition of eIF4E abrogates lymphocyte metabolic activation and differentiation, defining ACC1 as a key regulatory node. Our results demonstrate that translation is the mediator of T cell metabolism and indicate translation factors as targets for novel immunotherapeutic approaches.

WS.A5.02.03

Regulatory and conventional T cell transcription factors are reciprocally controlled during *Salmonella* infection

S. Clay, *A. Bravo Blas, D. Wall, M. MacLeod, S. Milling;*
Institutue of Infection, Immunity and Inflammation, Glasgow, United Kingdom.

Peripherally induced FoxP3$^+$ Tregs (pTregs) play an important role in controlling inflammation and maintaining homeostasis at mucosal sites. pTregs differentiate from conventional T cells and can express transcription factors (TFs) including T-bet, GATA3 and RORγT, markers used to identify T helper (Th) subsets. T-bet$^+$ Tregs can selectively suppress T-bet$^+$ Th1 cells but it is unclear whether Tregs expressing other TFs selectively inhibit corresponding Th subsets. It is also unclear whether this selective regulation influences T cell polarisation. To address these questions we use a *Salmonella enterica* serotype Typhimurium (STM) strain that allows characterisation of antigen specific and total T cells in lymphoid and mucosal sites. One week after oral infection, an increased proportion of Th17 cells are found in the colon, with a reduced proportion of Tbet$^+$ Th1 cells and increase in T-bet$^+$ Tregs. Two weeks post-infection this dynamic is switched to a Th1 bias, with a reduced proportion of RORγT$^+$ Th17 cells and increase in RORγT$^+$ Tregs. This reciprocity between conventional Th cells and Tregs expressing the same TFs occurs in the colon and caecum but not in draining lymph nodes. These findings are consistent with the hypothesis that pTregs shape T cell responses by selectively suppressing Th subsets at effector sites. To test this hypothesis we adoptively transfer Tregs expressing specific TFs into Treg-depleted recipients and assess the impact on the T cell response. This will reveal whether pTregs are capable of subset-specific regulation of Th cells, highlighting their potential utility for targeted therapeutic approaches.

WS.A5.02.04

CD28 costimulation and not TCR controls the effector functions of activated CD4 T cells

B. Soskic[1], *D. A. Glinos*[1], *D. M. Sansom*[2], *G. Trynka*[1];
[1]*Wellcome Sanger Institute, Cambridge, United Kingdom,* [2]*Institute of Immunity and Transplantation, University College London, London, United Kingdom.*

T cell response is initiated following interaction with antigen presenting cells. The T cell receptor (TCR) determines the specificity of the response and the CD28 costimulatory receptor helps to ensure that activation does not occur upon recognition of self-antigens. It is widely thought that CD28 is required for activation of naive and not memory T cells. Here, we used functional genomic assays to investigate the role of costimulation via CD28 on gene expression programmes in human naive and memory T cells. We demonstrate that T-helper differentiation, cytokine and chemokine expression increase in response to CD28 intensity in both naive and memory cells. Strikingly, we observe that cell cycle and cell division are sensitive to CD28 in memory cells, but under TCR control in naive cells, in contrast to the paradigm that memory cells are CD28-independent. Using a combination of chromatin accessibility and enhancer profiling, we demonstrate that interferon response elements (IRFs) and Blimp-1 motifs are enriched in naive and memory T cells in response to TCR. In contrast, memory cells initiate AP1 transcriptional regulation only when both TCR and CD28 are engaged, implicating CD28 as an amplifier of transcriptional programmes in memory cells. Lastly, we show that CD28-sensitive genes are enriched in autoimmune disease loci, pointing towards the role of memory cells and the regulation of T cell activation through CD28 in autoimmune disease development. This study provides new insights into the impact of TCR and CD28 in the activation of human naive and memory CD4 cells.

WS.A5.02.05

The genome organizer Satb1 is required for the development of T_H17 cells through regulation of IL-2 expression

M. Köhne[1,2], *D. Sommer*[2], *J. Schulte-Schrepping*[2], *L. Schmidleithner*[1,2], *K. Klee*[2], *J. Wißfeld*[2], *L. Holsten*[2], *K. Händler*[3], *M. Geyer*[4], *T. Kuhlmann*[5], *J. Alferink*[6], *C. Wickenhauser*[7], *J. L. Schultze*[2,3], *M. Beyer*[1];
[1]*Molecular Immunology in Neurodegeneration, German Center for Neurodegenrative Diseases (DZNE), Bonn, Germany,* [2]*Life & Medical Sciences Institute (LIMES), University of Bonn, Bonn, Germany,* [3]*PRECISE, Platform for Single Cell Genomics and Epigenomics, German Center for Neurodegenrative Diseases (DZNE), Bonn, Germany,* [4]*Institute of Innate Immunity, University of Bonn, Bonn, Germany,* [5]*Institute for Neuropathology, University of Münster, Münster, Germany,* [6]*Department of Psychiatry and Psychotherapy, University of Münster, Münster, Germany,* [7]*Institute for Pathology, Martin-Luther-University, Halle, Germany.*

T cells play an important role in host defense and tissue homeostasis. T-cell dysfunction is associated with multiple diseases such as increased susceptibility to pathogen infection, inflammatory and autoimmune diseases as well as cancer formation. Therefore, it is critical to identify the common underlying mechanisms governing T-cell differentiation and establish new approaches to influence their differentiation in disease settings.
Here we describe the genome organizer Special AT-rich-binding protein 1 (Satb1) as a critical regulator of T_H17-cell development. We could show that Satb1 is highly expressed during T_H17-cell differentiation and that a loss of Satb1 prevents T_H17-cell development. Furthermore, expression of Satb1 in CD4$^+$ T cells is required for the induction of autoimmune diseases, like experimental autoimmune encephalomyelitis (EAE) and inflammatory bowel disease. The formation of complex transcription factor networks, controlled through epigenetic modifications and chromatin reorganization, is required for specific T-cell function and linage commitment. Using transcriptional and epigenetic characterization of Satb1-deficient CD4$^+$ T cells, we could show that Satb1 mediates T_H17-cell development by preventing IL-2 expression early during T_H17-cell development. Satb1-dependent IL-2 regulation influences STAT proteins, the pioneer factors for T-cell differentiation, thus affecting T_H17-cell transcription factor network formation phenocopying Ahr ligation-mediated induction of T_H17-cell differentiation. In line with this, activation of Ahr signaling in Satb1-deficient CD4$^+$ T cells could rescue T_H17-cell differentiation.
Taken together, Satb1 is critical for the differentiation of T_H17 cells through epigenetic programming early during T_H17-cell development. Thus, Satb1 may pose a novel therapeutic target for the treatment of T_H17-cell driven autoimmune diseases.

WS.A5.02.06

Unique metabolic requirements for T helper 2 polarization by dendritic cells

L. Pelgrom[1], *A. Sergushichev*[2], *A. J. van der Ham*[1], *B. M. Winkel*[1], *M. Yazdanbakhsh*[1], *M. N. Artyomov*[3], *B. Everts*[1];
[1]*Leiden University Medical Center, Leiden, Netherlands,* [2]*ITMO University, Saint Petersburg, Russian Federation,* [3]*Washington University School of Medicine, St. Louis, United States.*

Dendritic cells (DCs) play a central role in the activation and polarization of T cell responses. We recently found that toll-like receptor signalling promotes a shift to glycolysis to support the anabolic demands of murine DC activation and effective priming of T cell responses. However, the metabolic requirements for polarization of distinct T helper cell (Th) responses by DCs, in particular Th2 responses, remain poorly defined. Based on unbiased global transcriptomic comparison of immature, Th1-, Th17- and Th2-priming human monocyte-derived DCs, we here report that suppression of genes involved in glycolysis is a key distinguishing feature of Th2-priming DCs. Consistent with these observations, in contrast to Th1- and Th17-priming DCs, Th2-priming DCs display low glycolytic rates and fail to increase glycolysis upon TLR stimulation. Importantly, blocking of glycolysis in DCs is sufficient to condition them for Th2 priming. Furthermore, based on unbiased analysis of integrated global metabolomic with transcriptomic data (CoMBI-T), we additionally identified an activated UDP-GlcNAc module in Th2-priming DCs, which points towards an important role for N- and/or O-glycosylation in Th2 priming by DCs. In line with these data, inhibition of O-linked GlcNAc transferase (OGT) impeded Th2 priming by DCs, without affecting their Th1- and Th17-priming capacity. Together, these findings suggest that DC-driven polarization of different T cell responses is dependent on the activation of distinct metabolic programs in DCs and highlights that metabolic manipulation of DCs could hold promise as a novel therapeutic approach to control immune-polarization in disease settings.

WS.A5.03 DC and tissue-derived cellular responses

WS.A5.03.01

Engulfment and active shuttling of mast cell granules boosts dendritic cell functions

J. Kotrba, *J. Dudeck, J. Froebel, A. Dudeck;*
Institute for Molecular and Clinical Immunology, Otto-von-Guericke University Magdeburg, Magdeburg, Germany.

Mast cells (MCs) are known as key effector cells of type I allergic reactions but also play an important role in host defense against pathogens. Despite increasing evidence for a critical impact of MCs on the induction of adaptive immunity, the underlying mechanisms are poorly understood. We therefore aimed at monitoring MC intercellular communication with neighboring dermal dendritic cells (DCs). We studied MC behavior and communication using intravital multiphoton microscopy of Mcpt5-Cre reporter mice. Moreover, we developed a strategy to stain the intracellular secretory MC granules *in vivo* inside the intact MCs allowing for the detection of MC degranulation. To assess the activation of DCs, intravital imaging was combined with flow cytometry, sorting and *ex vivo* functional assays. Here, we demonstrate using intravital imaging, that dermal DCs engulf the intact dense core secretory granules exocytosed by MCs upon LPS-induced skin inflammation. Subsequently, the engulfed MC granules are actively shuttled to skin draining lymph nodes (LNs) and finally degraded inside DCs within the lymphoid tissue. Most importantly, DCs bearing MC-granules show an advanced early migration to skin draining LN, a highly enhanced maturation and boosted T cell priming efficiency as compared to MC granule-negative DCs. Consequently, we highlight a unique feature of peripheral MCs to impact on lymphoid tissue borne adaptive immunity over distance by modifying DC functionality via the delivery of granule-stored mediators.

WS.A5.03.02

Cross-dressing of mast cells with MHC II from dendritic cells during skin Inflammation

J. Dudeck[1], *A. Medyukhina*[2], *J. Froebel*[1], *J. Kotrba*[1], *M. Figge*[2], *A. Dudeck*[1];
[1]*Institute for Molecular and Clinical Immunology, Otto-von-Guericke University Magdeburg, Magdeburg, Germany,* [2]*Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute Jena, Jena, Germany.*

Mast cells (MCs) and dendritic cells (DCs) are essential innate sentinels populating host-environment interfaces. Despite increasing evidence for a critical impact of MCs on DC functionality and the induction of adaptive immunity, the underlying mechanisms are poorly understood. Here, we studied the intercellular communication between MCs and dermal DCs during contact hypersensitivity (CHS) associated skin inflammation by means of longitudinal intravital multiphoton microscopy of MC/DC double reporter mice and extensive quantitative analysis. Further, the functional relevance of MC/DC interaction in T cell-driven CHS responses was analyzed by flow cytometry analysis and functional studies. We herein provide *in vivo* evidence that migratory DCs execute targeted cell-to-cell interactions with stationary MCs before leaving the inflamed skin to draining lymph nodes. During initial stages of skin inflammation, DCs dynamically scan MCs, whereas at a later stage, long-lasting interactions predominate. These innate-to-innate synapse-like contacts ultimately culminate in DC-to-MC molecule transfers including major histocompatibility complex class II (MHC II) proteins enabling subsequent ex vivo priming of allogeneic T cells with a specific cytokine signature. The extent of MHC II transfer to MCs correlates with their T cell priming efficiency. Importantly, preventing the cross talk by preceding DC depletion decreases MC antigen presenting capacity and T cell-driven inflammation. Consequently, we identify an innate intercellular communication arming resident MCs with key DC functions that might contribute to the acute defense potential during critical periods of migration-based DC absence.

WS.A5.03.03

CD86$^+$ antigen-presenting B cells are increased in solid cancers and induce tumor antigen-specific T cell responses

K. Wennhold[1], *M. Thelen*[1], *A. Lechner*[1], *H. Schlößer*[1,2], *M. von Bergwelt-Baildon*[3,4,5];
[1]*Center for Molecular Medicine Cologne, Cologne, Germany,* [2]*General-, Visceral- and Tumor Surgery, University Hospital Cologne, Cologne, Germany,* [3]*Department of Medicine III, University Hospital, LMU Munich, Munich, Germany,* [4]*German Cancer Consortium (DKTK), Heidelberg, Germany,* [5]*Comprehensive Cancer Center Munich (CCCM), Munich, Germany.*

B cell effector functions do not only include secretion of antibodies, but also presentation of antigen to T cells. Recently, a physiological B cell subset with strong immunostimulatory properties was described in humans. These antigen-presenting B cells (B_{ACP}) are characterized by a high expression of CD86 and downregulation of CD21. B_{ACP} are expanded following vaccination or under inflammatory conditions. We analyzed seven different tumor entities for the presence of B_{ACP} by flow cytometry and found increased percentages in lung adenocarcinomas, head and neck squamous cell carcinomas, colorectal cancer, esophageal-gastric cancers and renal cell carcinomas. Confocal microscopy demonstrated that CD86$^+$ B cells organize in tertiary lymphoid structures in the tumor microenvironment. Tumor antigen-specific B cells isolated from tumor-draining lymph nodes of cancer patients show increased percentages of B_{ACP}. Furthermore, we demonstrate a strong induction of tumor-specific T cell responses by B_{APC} using an antigen-specific fluorospot assay. Our results highlight the relevance of B_{ACP} as professional antigen-presenting cells in cancer.

WS.A5.03.04

Beta2-integrins restrict dendritic cell migratory phenotype through MRTFA/SRF signaling and a Syk-dependent epigenetic mechanism

C. Guenther[1], *M. Fusciello*[2], *M. Ilander*[1], *M. Sokolova*[3], *T. Savinko*[1], *L. Uotila*[1], *S. Yao*[4], *M. Moser*[5], *S. W. Morris*[6], *V. Cerullo*[2], *S. Tojkander*[4], *M. Vartiainen*[3], *S. C. Fagerholm*[1];
[1]*Department of Biosciences, University of Helsinki, Helsinki, Finland,* [2]*Department of Pharmacology, University of Helsinki, Helsinki, Finland,* [3]*Institute of Biotechnology, University of Helsinki, Helsinki, Finland,* [4]*Department of Veterinary Biosciences, University of Helsinki, Helsinki, Finland,* [5]*Department of Molecular Medicine, Max Planck Institute of Biochemistry, Munich, Germany,* [6]*Department of Hematology-Oncology, St. Jude Children's Research Hospital, Memphis, United States.*

Dendritic cells (DCs) are the classic antigen presenting cells of the immune system. DCs switch from an adhesive, phagocytic phenotype to a migratory phenotype in response to stimuli such as LPS[1] but also through unknown stimuli, resulting in spontaneous migration to lymph nodes. We have found that beta2-integrins regulate the migratory phenotype of DCs and their ability to induce strong immune responses[2]. We used a beta2-integrin knock-in mouse model, which lacks the beta2-integrin-kindlin-3 interaction (TTT/AAA-beta2-integrin KI mice) and thus has non-functioning beta2-integrins, to investigate how beta2-integrins restrict DC function. Interestingly, we found that beta2-integrin KI BMDCs displayed reduced adhesion and traction force generation but increased 3D migration speed *in vitro*. We show that RhoA activation and F-actin polymerization is abolished in beta2-integrin KI DCs, which leads to a failure of MRTF-A transcription factor to localize to the cell nucleus to co-activate genes with SRF. The integrin/RhoA/MRTFA/SRF pathway regulates DC adhesion, traction force generation and expression of chemokine receptors necessary for DC migration, eg CCR7. Furthermore, KI DCs display increased Syk activation and a Syk-dependent global increase in histone methylation (H3K4me3, characteristic of active genes). Inhibiting Syk led to a reduction of KI DC 3D migration speed, whilst inhibiting histone demethylases in WT DCs induced faster 3D migration. Furthermore, utilizing a B16OVA tumor model, we show that DCs expressing dysfunctional integrins induced increased tumour rejection *in vivo*. Thus, beta2-integrin-mediated adhesion to the extracellular environment restricts DC migration and DC-mediated tumor rejection *in vivo* through MRTFA/SRF signalling and a Syk-dependent epigenetic mechanism.

WS.A5.03.05

Expanded T-cell clones are present in the synovium before the clinical onset of rheumatoid arthritis

G. Balzaretti[1], *P. L. Klarenbeek*[1], *M. E. Doorenspleet*[1], *M. J. de Hair*[1], *B. C. van Schaik*[1], *R. E. Esveldt*[1], *M. G. van de Sande*[1], *D. M. Gerlag*[2], *A. H. van Kampen*[1], *F. Baas*[3], *P. Tak*[4], *N. de Vries*[1];
[1]*Academisch Medisch Centrum, Amsterdam, Netherlands,* [2]*Clinical Unit GlaxoSmithKline, Cambridge, United Kingdom,* [3]*Leiden University, Genome Diagnostics, Leiden, Netherlands,* [4]*GlaxoSmithKline, Stevenage, United Kingdom.*

Introduction: In healthy individuals with RA-specific autoantibodies the presence of expanded B-cell receptor (BCR) clones in peripheral blood (PB) accurately predicts who will develop arthritis in the short term. Following up on these observations, we investigated whether T-cell receptor beta (TCRβ) repertoire characteristics in PB and synovial tissue (ST) in this phase might also predict imminent onset of arthritis. Methods: Next-Generation Sequencing of the TCRβ repertoire was performed on 20 randomly selected individuals with elevated IgM-RF and/or ACPA levels. Ten individuals did not develop arthritis during at least 3 years of follow-up, and 10 individuals did. PB and ST samples were analysed during the *at-risk* phase and again after onset of arthritis.Results: In the *at-risk* phase the synovium is already characterized by expanded TCRβ clones, both in *at-risk* individuals that will and will not develop arthritis later. These clones persist during onset of arthritis. A higher impact of dominant TCRβ clones in synovial tissue at baseline was associated with longer time to arthritis (p=0.02). Conclusion: Expanded T-cell clones are present in the synovium in the *at-risk* phase regardless of future development of RA. They are maintained after onset of clinical disease. Combined with literature data, these observations show that T cell clones are already expanded in ST very early in disease, and suggest an overall regulatory role. Further studies are needed to characterize these clones.

WS.A5.03.06

Systems metabolic profiling reveals synergistic control of naive T cell priming by autophagy and mTOR

N. Franceco[1,2], *L. Papagno*[2], *A. Caputo*[1], *V. Appay*[2];
[1]*Department of Molecular Medicine, Padova, Italy,* [2]*CIMI Paris, Inserm U1135, UPMC, Hospital Pitié-Salpêtrière, Paris, France.*

96 Normal 0 false false false FR JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin:0cm; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:Calibri; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-ansi-language:FR;}
Introduction: The metabolic processes that regulate the fate of distinct T lymphocyte subpopulations remain poorly defined, especially in humans.
Materials and methods: We used a systems approach to characterize the basal and activation-induced energetic requirements of naive and phenotypically-defined subsets of memory CD4+ and CD8+ T cells. To test the importance of metabolic pathways on primary responses, we used an original model of in vitro priming of epitope-specific naïve $CD8^+$ T cells. Priming was performed in the presence of different drugs affecting metabolic pathways, and the expansion and phenotype of epitope-specific $CD8^+$ T cells assessed. **Results**: Profound metabolic differences were apparent as a function of lineage and differentiation status, both at rest and in response to stimulation via the T cell receptor (TCR). Of particular note, resting naive CD4+ and CD8+ T cells were largely quiescent, but rapidly upregulated diverse energetic pathways after ligation of surface-expressed TCRs. Moreover, autophagy and the mTOR-dependent glycolytic pathway were identified as critical mediators of antigen-driven priming in the naive CD8+ T cell pool, the efficiency of which was dampened by the presence of neutral lipids and fatty acids. **Conclusions**: These observations provided a metabolic roadmap of the T cell compartment in humans and revealed potentially selective targets for novel immunotherapies <!--EndFragment-->

WS.A6.01 Lessons learned from genetic defects

WS.A6.01.01

CLIP upregulation on B cells associates with multiple sclerosis onset and is governed by autoimmunity risk allele CLEC16A

L. Rijvers[1], *M. Meliefl*[1], *M. Stéphant*[1], *J. van Langelaar*[1], *R. M. van der Vuurst de Vries*[2], *A. F. Wierenga-Wolf*[1], *M. van Ham*[3], *R. Q. Hintzen*[1,2], *M. M. van Luijn*[1];
[1]*Department of Immunology, MS Center ErasMS, Erasmus MC, Rotterdam, Netherlands,* [2]*Department of Neurology, MS Center ErasMS, Erasmus MC, Rotterdam, Netherlands,* [3]*Department of Immunopathology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands.*

C-type lectin *CLEC16A* is located next to *CIITA* at a susceptibility locus for many autoimmune diseases, including multiple sclerosis (MS). We previously reported that CLEC16A is upregulated in MS and promotes the biogenesis of HLA-II peptide-loading compartments (MIIC) in myeloid antigen-presenting cells. Since T-cell activation by B cells is an important trigger of early MS, we questioned whether key members of the HLA-II pathway in B cells are associated with disease onset in MS patients, and how *CLEC16A* is involved in this process.
Blood B cells of clinically isolated syndrome (CIS) patients who rapidly develop MS (n=16) and clinically definite MS patients (n=15) were enriched for class II-associated invariant chain peptide (CLIP)-containing HLA-DR molecules and showed reduced CD74 (invariant chain) expression. Out of 15 autoimmunity-associated genes analyzed, the expression of *CLEC16A* correlated to CLIP/HLA-DR surface ratios (r=0.78) in human BLCL. *CLEC16A* was only triggered under CLIP-stimulating conditions *in vitro*, and was mainly expressed in $CLIP^{high}$ naive B-cell populations. Lentiviral shRNA-mediated knockdown of *CLEC16A* resulted in downregulation of CLIP-containing HLA-DR molecules and upregulation of CD74 on the plasma membrane (n=8). Moreover, CLEC16A-silenced B cells showed impaired BCR-mediated antigen uptake and extensive cytoplasmic scattering of MIIC, indicating that CLEC16A is a key regulator of human B-cell antigen processing and presentation.
These data demonstrate that HLA-II machinery in B cells is dysregulated in early MS and is functionally controlled by autoimmunity-associated *CLEC16A*. The abundance of CLIP-loaded HLA-II molecules on B cells mechanistically links to their escape from peripheral tolerance checkpoints during MS onset.

WS.A6.01.02

CD55deficientpatient PBMCs respond differentially to TLR, Inflammasome and patient exosome stimulation before and after Eculizumab therapy

G. G. Kaya[1], *M. Yildirim*[1], *I. Evcili*[1], *N. Bozbeyoglu*[1], *I. C. Ayanoglu*[2], *A. Ozen*[3], *S. Sari*[4], *B. Dalgic*[4], *M. Gursel*[2], *I. Gursel*[1];
[1]*Bilkent University, Ankara, Turkey,* [2]*Middle East Technical University, Ankara, Turkey,* [3]*Marmara University, Istanbul, Turkey,* [4]*Gazi University, Ankara, Turkey.*

CD55 is a membrane bound protein whose deficiency has been recently defined as hyperactivation of complement, angiopathic thrombosis, and protein-losing enteropathy (CHAPLE syndrome). It inhibits classical and alternative complement pathways while it acts and mediates activity of leukocytes via CD97 engagement. Herein, $CD55^{-/-}$ immune cell responses in the presence of TLR and Inflammasome ligand stimulation and exosome incubation was studied. Fresh plasma and PBMCs of CHAPLE patients (N=7) before and after Eculizumab therapy (BT & AT, respectively) were obtained. PBMCs were stimulated with various ligands such as p(I:C), LPS, R848, CpG ODNs, cGAMP Nigericin and BT and AT exosomes. Responses were assessed for IL-1β, IL-8, IL-10, IP-10, IFNγ and IFNα levels from cell supernatants. Results revealed that IL-8 and IP10 levels from plasma and stimulated BT cells were higher and AT exosomes induced PBMCs to secrete elevated IL-8 levels compared to AT exosomes. The IFNγ secretions BT and AT to PMA, R848 and cGAMP showed higher levels compared to healthy cells. Moreover, AT exosomes of patients induced substantially higher IFNγ levels. IL-10 secretion in response to TLR2, 4 and 7 were found to be lower even AT but significantly increased compared to BT measurements. Furthermore, AT exosomes induced healthy PBMCs to secrete less IL-10 compared to BT exosomes. Spontaneous NETotic tendencies of patient neutrophils significantly subsided after Eculizumab therapy. Healthy or AT exosomes compared to BT exosomes induced much lesser NET formation from both healthy or AT neutrophils. Consequently, $CD55^{-/-}$ patients PBMCs regain healthy donor-like character following Eculizumab therapy.

WS.A6.01.03

A Th17 cell specific migration defect provides protection from EAE in DOCK8 deficient mice

A. S. Wilson[1], *H. Law*[1], *C. B. Knobbe-Thomsen*[2], *C. J. Kearney*[3], *J. Oliaro*[3], *C. Binsfeld*[4], *G. Burgio*[1], *L. Starrs*[1], *D. Brenner*[4], *K. L. Randall*[1], ***A. Brüstle***[1];
[1]*The Australian National University, Canberra, Australia,* [2]*Heinrich Heine University Düsseldorf, Düsseldorf, Germany,* [3]*Peter MacCallum Cancer Centre, Melbourne, Australia,* [4]*LIH Luxembourg Institute of Health, Luxembourg, Luxembourg.*

Mice lacking functional dedicator of cytokinesis 8 (DOCK8), an immune cell-expressed guanine exchange factor for Cdc42, have recently been described as resistant in the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS). The IL-17 producing T helper 17 (Th17) cells are seen as the major mediator of central nervous system (CNS) inflammation in this model. We here investigate for the first time the role of DOCK8 in Th17 mediated CNS inflammation.
Contrary to expectation, we observed an elevation of Th17 cells (CD4+ IL17a producing cells) in two different DOCK8 mutant mouse strains in the steady state. In vitro T helper cell differentiation revealed that the elevated Th17 cell population was not based on a T cell intrinsic differentiation bias. Th17 cells remain elevated systemically during an episode of EAE but the cellular infiltration in the CNS is reduced in these mice, out of proportion to the systemic balance in Th1 and Th17 cells. DOCK8 deficient Th17 cells expressed normal Th17 cell specific CCR6 levels and migrated towards chemokine gradients in transwell assays, but adoptive transfers of Th1 and Th17 cells in EAE affected mice indicated a Th17 cell specific migration defect in the absence of functional DOCK8. Here we show for the first time a cell intrinsic mediator of migration that specifically affects the pathogenic Th17 cell subset responsible for neuroinflammation in this model further elucidating the mechanism of resistance of DOCK8 mutant mice to EAE.

WS.A6.01.04

Consequences of an IL2RA locus duplication in a very early onset inflammatory bowel disease patient

M. E. Joosse[1], *F. Charbit-Henrion*[2], *R. C. Raatgeep*[1], *D. J. Lindenbergh-Kortleve*[1], *L. M. Costes*[1], *S. Nugteren*[1], *S. Veenbergen*[1], *V. Malan*[2], *J. K. Nowak*[3], *M. Mearin*[4], *J. C. Escher*[1], *N. Cerf-Bensussan*[2], *J. N. Samsom*[1];
[1]*Erasmus MC, Rotterdam, Netherlands,* [2]*Institute National de la Santé et de la Recherche (INSERM), Institute Imagine and Université Paris-Descartes, Paris, France,* [3]*Poznan University of Medical Sciences, Poznan, Poland,* [4]*Leiden University Medical Center, Leiden, Netherlands.*

Rare single genetic mutations can predispose to very early onset inflammatory bowel disease (VEO-IBD). Here, we identify a *de novo* duplication of the 10p15.1 chromosomal region, including the *IL2RA* locus, in a 2-year-old patient presenting with therapy-resistant VEO-IBD that was brought into remission by subtotal colectomy. As IL-2 is important for T-cell survival and proliferation, we hypothesized that the *IL2RA* duplication results in aberrant T-cell function. Indeed, after subtotal colectomy and without medication, the patient still had an increased CD4 to CD8 ratio in peripheral blood and an increased CD25 expression on circulating effector memory, Foxp3^{+} and Foxp3^{neg} CD4^{+} T cells. Isolated patient CD4^{+} T cells were intrinsically activated as evidenced by increased STAT5 phosphorylation, proliferation and Ki67 expression. In agreement with increased CD25 surface expression, patient CD4^{+} T cells were more sensitive to low-dose IL-2 compared to healthy control cells. The colon, but not the unaffected duodenum, was infiltrated with proliferating CD3^{+} cells in the lamina propria and epithelial layer. Inflamed colonic tissue contained numerous Tbet^{+} cells and expressed high levels of *IFNG* mRNA. Crucially, inhibiting IL-2 signaling with the JAK1/3 inhibitor tofacitinib ablated IFNγ secretion and restored normal CD25 expression by CD4^{+} T cells. In conclusion, the patient's immune system exhibits constitutive activation of the CD25-phosphoSTAT5 pathway leading to hyper-proliferative CD3^{+} T cells predisposing to a T-cell driven pancolitis. These findings shed new light on the role of IL-2 in intestinal homeostasis and directs further studies to examine the functional consequences of *IL2RA* genetic variation in IBD patients.

WS.A6.01.05

Coincidence of a novel *NFKB1* mutation with a Crohn-associated *NOD2* rare variant in a patient may explain her profound common variable immunodeficiency (CVID) phenotype with unusually severe gastrointestinal manifestations

M. Martinez-Gallo[1], *R. Dieli-Crimi*[2], *C. Franco-Jarava*[2], *M. Antolin*[3], *L. Blasco*[3], *P. Paramonov*[3], *A. Álvarez Fernández*[4], *X. Molero*[5], *J. Velázquez*[5], *A. Martín-Nalda*[6], *R. Pujol-Borrell*[1], *R. Colobran*[1];
[1]*Immunology Division, Hospital Universitari Vall d'Hebron (HUVH), Vall d'Hebron Research Institute (VHIR), Department of Cell Biology, Physiology and Immunology, Autonomous University of Barcelona (UAB)., Barcelona, Spain,* [2]*Immunology Division. Hospital U. Vall d'Hebron, Barcelona, Spain,* [3]*Area of Clinical and Molecular Genetics. Hospital U. Vall d'Hebron, Barcelona, Spain,* [4]*Pneumology Department. Hospital U. Vall d'Hebron, Barcelona, Spain,* [5]*Department of Digestive Diseases Hospital U. Vall d'Hebron, Barcelona, Spain,* [6]*Pediatric Infectious Diseases and Immunodeficiencies Unit (UPIIP), Hospital Universitari Vall d'Hebron., Barcelona, Spain.*

Monoallelic loss-of-function mutations in *NFKB1* have been recently recognized as the most frequent monogenic cause of common variable immunodeficiency (CVID). The prototypic clinical phenotype of NFKB1 deficient patients included common CVID features as hypogammaglobulinemia and sinopulmonary infections plus other very diverse complications. Here we report a patient with a profound CVID phenotype and severe gastrointestinal manifestations. Using a custom panel of 323 genes causative of primary immunodeficiencies and massive parallel sequencing we identified a novel monoallelic loss-of-function mutation in *NFKB1*: a 1-bp deletion in exon 12 (c.1149delT) leading to a frameshift and a premature stop codon (p.Gly384Glu*48). Interestingly, we also found a rare missense variant in *NOD2* known to be associated to Crohn's disease (p.His352Arg). Our patient presented hypogammaglobinemia with of low number of B most of which are naïve. The most remarkably findings included a marked skewing towards a Th1 tbet+ phenotype in peripheral blood T cells and an excessive production of proinflammatory cytokines (IL-1beta, TNF-alfa and IFN-gamma). The 6-year old patient's daughter, carrier of the *NFKB1* mutation, remained clinically asymptomatic but started to show changes at cellular and molecular level. Data here presented constitutes an example this case of NFKB1 deficiency a combined immunodeficiency with a hyperinflammatory state and a glimpse of the preclinical period.

WS.A6.01.06

Tetratricopeptide repeat domain 7 a regulates haematopoietic stem cell functions by controlling the stress-induced response

C. Leveau[1], *M. El-Daher*[1], *N. Cagnard*[1,2], *A. Fischer*[1,3,4], *G. de Saint Basile*[1,5], *F. Sepulveda*[1];
[1]*Imagine Institute, INSERM UMR 1163. Université Paris Descartes -Sorbonne Paris Cité, Paris, France,* [2]*SFR Necker, INSERM US24/CNRS UMS 3633, Paris, France,* [3]*Assistance Publique-Hôpitaux de Paris, Hôpital Necker-Enfants Malades, Immunology and Pediatric Hematology Department, Paris, France,* [4]*Collège de France, Paris, Austria,* [5]*Assistance Publique-Hôpitaux de Paris, Hôpital Necker-Enfants Malades, Centre d'Etudes des Déficits Immunitaires, Paris, Austria.*

The molecular machinery that regulates the balance between self-renewal and differentiation properties of hematopoietic stem cells (HSCs) has yet to be characterized in detail. We sought to determine the role of tetratricopeptide repeat domain 7 A (Ttc7a) protein, a putative scaffold protein, in HSCs biological functions. We found that Ttc7a acts as an intrinsic regulator of the proliferative response and the self-renewal potential of murine HSCs *in vivo.* Loss of Ttc7a consistently enhanced the HSCs' competitive repopulation ability and their intrinsic capacity to replenish the hematopoietic system after serial cell transplantations, relative to wild-type cells. To gain insights into the molecular mechanisms, we performed RNAseq study on control and Ttc7a-deficient HSCs. Ttc7a-deficient cells exhibit a different transcriptomic profile for a set of genes controlling the cellular response to stress, which was associated with increased proliferation in response to chemically induced stress *in vitro*and myeloablative stress *in vivo*. Our results therefore reveal a previously unrecognized role of Ttc7a as a critical regulator of the HSCs stemness. This role is related, at least in part, to regulation of the endoplasmic reticulum stress response.

WS.B1.01 Immune checkpoints in anti-tumor therapy

WS.B1.01.01

Impact of tumor heterogeneity on response to immune checkpoint inhibition

S. Sánchez Alonso[1], *G. Setti Jeréz*[2], *M. Arroyo Correa*[1], *T. Hernandez Paredero*[2], *A. Alfranca González*[1];
[1]*Hospital Universitario de La Princesa, Madrid, Spain,* [2]*Universidad Autónoma de Madrid, Madrid, Spain.*

Lung cancer has emerged as the leading cause of death from cancer among men and women worldwide in the past decades. Several factors are involved in regulating tumor growth, and among them host immune system has an important role in modulating tumor behavior. Specially, tumor escape from immunosurveillance and its progression from a latency state are strongly affected by immune system function, which suggests the existence of heterogeneous subpopulations composed by tumor cells with different immunogenic potential. Particularly, generation of an immunosuppressive microenvironment is widely recognized to be essential in tumor escape. Thus, novel therapies focused on blockade of inhibitory checkpoints, such as anti-PD1 antibodies, are being developed to improve immune response against tumor.
We have analyzed the influence of intratumoral heterogeneity in the immune response to immunotherapy in non-small cell lung cancer (NSCLC) to identify potential conditioning factors of response to treatment. For this, we have established a syngeneic mouse model of NSCLC marked with RGB lentiviral vectors for multicolor cell tracking, to analyze clonal subpopulations in tumors treated with anti-PD1 or control antibodies. This has allowed us to track different clonal subpopulations derived from initial tumor and isolate treatment resistant subsets by cell sorting, some of which were re-inoculated to analyze the behavior of each one individually. We have studied tumor progression, host immune response and gene expression profile in individual clonal subpopulations derived from anti-PD1- and control-treated NSCLC.

WS.B1.01.02

Rational therapeutic modulation of the tumour microenvironment sensitizes cancers to immune checkpoint blockade

R. M. Zemek[1,2], *E. De Jong*[3], *W. L. Chin*[1,2], *V. Fear*[2], *C. Forbes*[2], *T. Casey*[2], *A. R. Forrest*[4], *D. O'Muiri*[4], *M. Small*[5], *A. Zaitouny*[5], *M. J. Millward*[6,1], *A. K. Nowak*[6,1,2], *T. Lassman*[7], *R. A. Lake*[8,2], *A. Bosco*[7], *W. J. Lesterhuis*[8,2];
[1]*School of Medicine, University of Western Australia, Perth, Australia,* [2]*National Centre for Asbestos Related Diseases, Perth, Australia,* [3]*Telethon Kids Institute, Perth, WA, Australia,* [4]*Harry Perkins Institute for Medical Research, Perth, Australia,* [5]*School of Mathematics, University of Western Australia, Perth, Australia,* [6]*Dept of Medical Oncology, Sir Charles Gairdner Hospital, Perth, Australia,* [7]*Telethon Kids Institute, Perth, Australia,* [8]*School of Biomedical Sciences, University of Western Australia, Perth, Australia.*

Background: It is not well understood what molecular events contribute to an effective response to immune checkpoint blockade (ICB). Numerous combinations of drugs with ICB are currently being trialled with limited empirical preclinical evidence of efficacy. Predictive biomarkers and a rational approach to improve efficacy are therefore urgently needed.
Methods: Using inbred mouse strains inoculated bilaterally with monoclonal cancer cell lines, treatment with ICB results in a clear symmetric, yet dichotomous response, allowing us to sample entire tumours from one side and correlate findings with response, knowing the future outcome from the contralateral tumour. We used bulk RNAseq, single cell RNAseq and flow cytometry to characterise the pre-treatment tumour microenvironment of responding versus non-responding tumours. Findings were validated using publicly available RNAseq datasets of patients treated with ICB, and through in vivo pharmacologically targeting in murine cancer models.
Results: Responders had greater NK cell infiltrate and upregulation of NK associated genes. In addition, responders displayed an inflammatory gene expression signature driven by STAT1, which was corroborated in a dataset of patients with diverse cancers treated with ICB. We identified key positive and negative upstream regulators that were predicted to induce the response-associated signature. Pre-treatment of difficult-to-treat mouse tumour models, using a combination of therapeutics targeting these upstream regulators resulted in full cures when they were subsequently treated with immune checkpoint blockade.
Conclusion: We identified a pre-treatment STAT1-driven gene signature associated with response. Phenocopying this response-associated gene expression profile by rational pre-treatment of the tumour microenvironment sensitizes tumours to ICB.

WS.B1.01.03

Molecular recalibration of PD-1+ antigen-specific T cells as immunotherapy for Hepatocellular carcinoma

I. Otano[1,2], *D. Escors*[1,3], *A. Schurich*[4], *H. Singh*[4], *F. Robertson*[5], *B. Davidson*[5], *G. Fusai*[5], *F. A. Vargas*[6], *Z. Tan*[7], *J. Aw*[7], *N. Hansi*[8], *P. Kennedy*[8], *S. Xue*[9], *H. Stauss*[9], *A. Bertoletti*[2,10], *A. Pavesi*[2], *M. K. Maini*[1];

[1]*Division of Infection and Immunity, UCL, London, United Kingdom,* [2]*Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore, Singapore,* [3]*Navarrabiomed-Biomedical Research Centre, IdiSNA, Pamplona, Spain,* [4]*University College of London, London, United Kingdom,* [5]*Department of Surgery and Interventional Science, UCL, London, United Kingdom,* [6]*Institute of Cancer Research, UCL, London, United Kingdom,* [7]*Institute of Molecular and Cell Biology, Agency for Science, Technology and Research,, Singapore, Singapore,* [8]*Centre for Immunobiology, Blizard Institute, Bart's and the London School of Medicine and Dentistry, QMUL,, London, United Kingdom,* [9]*Institute of Immunity and Transplantation, UCL, London, United Kingdom,* [10]*Emerging Infectious Diseases Program, Duke-NUS Graduate Medical School, Singapore, Singapore.*

Introduction: Checkpoint inhibitors and adoptive cell therapy provide promising options for treating solid cancers such as hepatitis B-related hepatocellular carcinoma but have limitations. We tested the potential to combine advantages of each approach, genetically re-programming T cells specific for viral/tumour antigens to overcome exhaustion by down-modulating the co-inhibitory receptor PD-1. **Methods:** We developed a novel lentiviral transduction protocol to achieve preferential targeting of endogenous low-frequency or TCR-redirected antigen-specific CD8 T cells for shRNA knockdown of PD-1 and tested functional consequences for anti-tumour immunity in 2D and 3D cultures. **Results:** Antigen-specific CD8 T cells transduced with LV-shPD-1 consistently had a marked reduction in PD-1 compared to those transduced with a control lentiviral vector. PD-1 could also be down-modulated on liver-resident or T cell receptor (TCR)-redirected T cells. PD-1 knockdown of human T cells rescued anti-tumour effector function and promoted killing of hepatoma cells in a 3D microdevice recapitulating the pro-inflammatory PD-L1^{hi} liver microenvironment.
However, upon repetitive stimulation, PD-1 knockdown drove T cell senescence and induction of other co-inhibitory pathways. **Conclusion:** We provide proof-of-principle that T cells with endogenous or genetically engineered specificity for HCC viral antigens can be targeted for functional genetic editing. We show that PD-1 knockdown enhances immediate tumour killing but is limited by compensatory engagement of alternative co-inhibitory and senescence programmes upon repetitive stimulation. **Grants:** EASL postdoctoral fellowship, WT Senior Investigator Award.

WS.B1.01.04

CD47-SIRPa checkpoint blockade involves kindlin3-dependent enhancement of CD11b/CD18-integrin affinity and cytotoxic synapse formation

P. Bouti[1], *H. Matlung*[1], *M. van Houdt*[1], *P. Verkuijlen*[1], *K. Franke*[1], *T. W. Kuijpers*[1,2], *T. K. van den Berg*[1,3];
[1]*Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands,* [2]*Academic Medical Centre, Amsterdam, Netherlands,* [3]*Department of Molecular Cell Biology and Immunology, VU medical center, Amsterdam, Netherlands.*

Recently, we established that neutrophils kill antibody-opsonized tumour cells by a novel cytotoxic process that we have termed *trogoptosis*. This previously unknown killing mechanism involves trogocytosis (from Greek *trogo*, gnaw), where fragments of target cell membrane are actively taken up by the neutrophil, thereby disrupting the target cell plasma membrane and killing the cancer cells. Trogocytosis and subsequent killing is strictly dependent on antibody-opsonization of the tumour cells, neutrophil Fcγ-receptor signalling and CD11b/CD18 integrin-dependent cytotoxic synapse formation. Furthermore, it is promoted by CD47-SIRPα checkpoint inhibition. Here, we present evidence that CD47-SIRPα interactions act by controlling the initial stage of the killing process i.e. the CD11b/CD18-dependent cytotoxic synapse formation. In particular, CD47-SIRPα interactions negatively regulated the CD11b/CD18 inside-out activation that occurred as a consequence of Fc-receptor signalling in neutrophils. Moreover, the inhibitory effect acted via the integrin-associated protein kindlin-3, as demonstrated, amongst other things, by using neutrophils from rare LAD-III patients that have mutations in FERMT3 and lack kindlin-3 expression. Collectively, these findings demonstrate that CD47-SIRPα interactions control a kindlin-3-dependent pathway of CD11b/CD18-integrin activation, and that targeting the CD47-SIRPα checkpoint primarily improves integrin activation, and therefore also the resultant cytotoxic synapse formation, trogocytosis and killing during neutrophil ADCC towards cancer cells.

WS.B1.01.05

HVEM as a new checkpoint blockade for cancer immunotherapy

P. KC[1], *S. Brunel*[1], *N. Aubert*[1], *D. Olive*[2], *G. Marodon*[1];
[1]*Sorbonne Université - Centre d'immunologie et des maladies infectieuses-Paris, UMRS 1135, Paris, France,* [2]*Aix-Marseille Universités, Inserm, CNRS, CRCM, Institut Paoli-Calmettes, Marseille, France.*

Expression of the co-inhibitory molecule Herpes Virus Entry Mediator (HVEM) has been identified in a wide range of cancers and its expression level might be inversely correlated with patient survival.
HVEM positive tumor cells could inhibit the immune response through co-inhibitory molecules BTLA or CD160, the main HVEM ligands expressed by human T lymphocytes.
To release the anti-tumor immune response, a monoclonal antibody (mAb) targeting this immune checkpoint was evaluated in NSG mice, genetically deficient for T, B and NK cells. NSG mice grafted with PBMC and PC3, a human prostate cell line expressing HVEM, showed a reduced tumor growth with anti-HVEM therapy whereas no effect was observed with another prostate cancer cell line not expressing HVEM. TILs had an increased proportion or proliferation of CD8+ T cells, suggesting that the mAb improved anti-tumor immunity. Albeit to a lesser extent, reduction in tumor growth was also observed in mice without PBMC. Depletion experiments with anti-Gr1 antibody indicated a role for myeloid cells on tumor growth. Moreover, co-culture of NSG macrophages and PC3 showed an increase in tumor mortality with the anti-HVEM.
These results suggest that the anti-HVEM mAb might directly target the tumor and recruits myeloid cells to induce cell death while at the same time, it might release local immunosuppression by preventing the inhibitory HVEM/BTLA interaction to occur. Thus, HVEM, by its dual effect on tumors and T lymphocytes, is a promising target for cancer immunotherapy.

WS.B1.01.06

Gastro-intestinal Stromal Tumors show enhanced numbers of checkpoint-negative CD8 T-cells

Y. Klaver, M. Rijnders, A. Oostvogels, R. Wijers, M. Smid, D. Grunhagen, C. Verhoef, S. Sleijfer, ***C. Lamers****, R. Debets;*
Erasmus MC Cancer Institute, Rotterdam, Netherlands.

Soft Tissue Sarcoma (STS) is a relatively rare type of cancer with over 50 different subtypes that can originate from fat, muscle, nerves, fibrous, endothelial, or deep skin tissues. To explore whether STS subtypes show differential sensitivity to immune therapies, we analyzed quantity, immune phenotype and TCR diversity of tumor infiltrating lymphocyte (TIL) as well as immunogenicity of gastrointestinal stromal tumors (GIST), leiomyosarcoma (LMS), liposarcoma (LPS), myxofibrosarcoma (MFS), and pleomorphic sarcoma (PMS). Fresh resection specimen were processed for in situ stainings and single cell flowcytometry with a focus on CD8 T-cell differentiation and surface expression of immune checkpoints. STS DNA and RNA sequencing data were processed in silico to assess mutational load, TCR diversity, frequencies of immune cells, and antigen presentation and expression of checkpoints and their corresponding ligands. GIST, MFS and PMS were observed to have relatively high numbers of CD8 TILs with highest fractions of differentiated CD8 T-cells in GIST and LPS. These CD8 TILs generally expressed the checkpoints PD-1, LAG3 and TIM3, with highest co-expression in MFS and PMS and, unexpectedly, nearly absent co-expression in GIST. Interestingly, when analyzing TCR-Vb sequences, the fractions of dominant clones were lowest in GIST and LPS. Interrogation of Next Generation Sequencing data revealed that numbers and checkpoint expression levels of CD8 TILs relate to immune evasive mechanisms, in particular with respect to intra-tumoral T-cell activation. In conclusion, STS subtypes differ in quantity and quality of CD8 TILs, making these subtypes amenable to different forms or combinations of immune therapy.

WS.B1.02 Novel targets in anti-cancer immune therapy

WS.B1.02.01

BAFF-R as a novel therapeutic target for Acute Lymphoblastic Leukemia

R. Parameswaran*, Y. Vicioso;*
Case western reserve university, Cleveland, United States.

Introduction: Acute Lymphoblastic Leukemia (ALL) is the most common childhood cancer. Drug resistance and relapse are two major problems in ALL. Pre-B ALL cells express B-cell activating factor receptor (BAFF-R) on their surface, which is not present on normal pre-B cells. **Results:** We observed that BAFF-R expression was maintained in drug resistant and relapse pre-B ALL cells. We used an anti-BAFF-R antibody optimized for antibody dependent cellular cytotoxicity (ADCC) to enhance NK cell mediated killing of drug resistant and relapsed ALL cells, which are difficult to treat by conventional chemotherapy. We found that anti-BAFF-R antibody enhanced NK cell mediated killing of drug resistant and relapse ALL cells *in vitro.* Early treatment with anti-BAFF-R antibody and NK cells significantly reduces disease burden in xenograft ALL models. However, in advanced disease, the efficacy of anti-BAFF-R antibody to mediate ADCC is reduced. ALL cells are known to produce TGF-beta, that causes NK cell dysfunction. We found that co-culturing ALL cells with NK cells lead to a decrease in FCγ-RIII expression on NK cells. Since FCγ-RIII is essential for ADCC, we evaluated the effect of EW-7197, a potent TGF-beta receptor I inhibitor, to enhance ADCC killing of ALL cells. We found that neutralizing TGF-beta receptor I on NK cells, using EW-7197, prevented ALL and TGF-beta-induced downregulation of FCγ-RIII expression in NK cells and improved *in vitro* and *in vivo* killing of ALL cells. **Conclusion:** Treatment with BAFF-R antibody, NK cells and EW-7197 may eradicate relapse and drug resistant pre-B ALL.

WS.B1.02.02

Investigation of novel immunotherapeutic targets for colorectal cancer

D. Shinko[1], *H. McGuire*[2,3], *T. Ashhurst*[3,2], *S. Clarke*[4], *S. Byrne*[5,6], *K. Charles*[1];
[1]*Discipline of Pharmacology, The University of Sydney, Sydney, Australia,* [2]*Discipline of Pathology, The University of Sydney, Sydney, Australia,* [3]*Ramaciotti facility for Systems Biology, The University of Sydney, Sydney, Australia,* [4]*Royal North Shore Hospital, Sydney, Australia,* [5]*The Westmead Institute for Medical Research, Sydney, Australia,* [6]*Discipline of Infectious Diseases and Immunology, The University of Sydney, Sydney, Australia.*

An enhanced understanding of the immune-tumour interaction has led to significant clinical benefit in the use of immunotherapy in cancer. In colorectal cancer, however, a lack of understanding of the immune cell complexity means that the correct drug targets for exploitation remain elusive. Our aim was to investigate the immune phenotype and inflammatory signalling pathways in colorectal cancer patients undergoing chemotherapy to identify novel immunotherapeutic targets. Using a 35-marker mass cytometry panel, we quantified 7 major circulating immune cell types and over 20 subtypes in 10 advanced colorectal cancer patients and 9 healthy volunteers. We found significant difference in major B cell populations and sub-populations of T cells (including regulatory T cells, central memory T helper cells and effector memory cytotoxic T cells) between the patients and the healthy volunteers. Further interrogation of B cell sub-populations are underway. We also quantified nine phosphorylated intracellular signalling markers. At baseline, results show that pP38 and pSTAT3 are significantly decreased in patients across the majority of immune cells whereas pSTAT5 is increased. Results also show that phosphorylated markers, such as pERK, can be activated following a cycle of chemotherapy and remain activated throughout the therapy. Relationships between immune profiles and clinical outcomes are currently being explored. The use of mass cytometry has allowed the investigation of the immune profile of CRC patients and potential novel immunotherapeutic targets have been identified to improve clinical outcomes.

WS.B1.02.03

IL-1R8 is a novel checkpoint regulating anti-tumor and anti-viral activity of NK cells

M. Molgora[1], *E. Bonavita*[2], *A. Ponzetta*[1], *F. Riva*[2], *M. Barbagallo*[2], *S. Jaillon*[1], *B. Popovic*[3], *G. Bernardini*[4], *E. Magrini*[2], *F. Gianni*[2], *S. Zelenay*[5], *S. Jonjic*[3], *A. Santoni*[4], *C. Garlanda*[1], *A. Mantovani*[1];
[1]*Humanitas University, Pieve Emanuele, Italy,* [2]*Humanitas Research Hospital, Rozzano, Italy,* [3]*University of Rijeka, Rijeka, Croatia,* [4]*Università di Roma "La Sapienza", Roma, Italy,* [5]*Cancer Research UK, Manchester, United Kingdom.*

IL-1R8 is an Interleukin-1 receptor family member that acts as a negative regulator of IL-1 family receptor and TLR signaling. Both murine and human NK cells express high levels of IL-1R8 but its functional role in this cell type has not been described so far. Expression analysis showed that IL-1R8 was acquired during differentiation in human and murine NK cells. IL-1R8 deficiency in the mouse was associated with enhanced NK cell maturation and activation. IL-18, which is a key regulator of NK cell activities and can be targeted by IL-1R8, was responsible for this phenotype. To assess the role of IL-1R8 in NK cells in pathology, we used models of MCA-induced lung metastasis, colon cancer-derived liver metastasis and DEN-induced hepatocellular carcinoma. The number and dimension of liver and lung metastasis and the liver disease severity were significantly reduced in *Il1r8*$^{-/-}$ mice. The depletion of NK cells in these models totally abrogated the protection observed in *Il1r8*$^{-/-}$ mice. Finally, we investigated the role of IL-1R8 in NK cell antiviral activity, in a model of MCMV infection. *Il1r8*$^{-/-}$ mice controlled the virus more efficiently in the liver and the protection was associated with enhanced NK cell degranulation and IFN-γ production. The adoptive transfer of *Il1r8*$^{-/-}$ NK cells conferred protection in both metastasis and viral infection models. IL-1R8 plays a non-redundant role in the regulation of NK cell development and effector functions by tuning IL-18-dependent activities. IL-1R8 therefore emerges as a crucial regulator of NK cell antitumoral and antiviral potential.

WS.B1.02.04

Glycan modified vesicles as a targeted and personalized vaccination strategy for the induction of anti-tumor immunity

S. K. Horrevorts*, D. A. Stolk, S. J. van Vliet, T. D. de Gruijl, A. A. van de Loosdrecht, Y. van Kooyk;*
Vu university medical center, Amsterdam, Netherlands.

Effective immunotherapies should boost existing or elicit *de novo,* tumor-specific immune responses. Challenges in vaccine development are the choice of suitable antigens and targeting of these antigens to antigen presenting cells (APCs). We developed a new personalized vaccination approach, which encompasses apoptotic vesicles derived from the patients tumor and glyan modification, to allow targeting to APCs for the induction of tumor specific T cells. Apoptotic vesicles derived from the patients tumor contain both tumor associated antigens (TAAs) and neo-antigens, making them a potent source for vaccination. For the efficient targeting of the vesicles to APCs we modified their glycocalyx, resulting in the surface expression of high mannose glycan structures. These glycans are the natural ligand of the DC specific C-type lectin receptors DC-SIGN and Langerin, expressed on skin dermal DCs (dDCs) and Langerhans cells (LCs), respectively. Using *ex vivo* skin explants as a model for intradermal vaccination in humans, we are able to specifically target apoptotic vesicles to dDCs and LCs, thereby significantly increasing vesicle uptake, resulting in an enhanced antigen presentation to $CD8^+$ T cells. In conclusion we developed a novel personalized vaccine strategy were we combine patient derived tumor vesicles with glycan modification to efficiently target (neo-) antigens to APCs, for the induction of anti-tumor immune responses.

WS.B1.02.05

Heme as a modulator of the therapeutic efficacy of an anti-cancer antibody

A. Tavares-Kanyavuz*, A. Marey-Jarossay, S. Lacroix-Desmazes, J. Dimitrov;*
SorboINSERM UMR_S 1138, Centre de Recherche des Cordeliers, 75006 Paris, France.

Introduction: Polyreactive antibodies have potential to bind to multiple structurally unrelated antigens. Some apparently monoreactive antibodies like Rituximab can acquire polyreactivity post-translationally by contact with heme. Rituximab (anti-CD20) is used for the treatment of different B-cell malignancies and among these diseases some can be accompanied by hemolysis. The main goal of this study is to characterize the effect of heme on therapeutic efficacy of anti-CD20 antibodies. Materials and Methods: We performed *in vitro* studies with human B-cell lymphoma cell lines, to characterize the polyreactivity and cytotoxicity of Rituximab after heme-exposure. Then, we evaluated the impact of heme binding on the therapeutic activity of Rituximab using a murine B-lymphoma model. Results: Rituximab acquires a large gain of reactivity after exposure to heme, while retaining its capacity to bind to its cognate antigen. Induction of polyreactivity improves therapeutic efficacy of Rituximab *in vitro* and *in vivo*. Conclusions: We documented an important role of the induced polyreactivity in the function of anti-CD20 antibodies. This phenomenon may occur in patients treated with Rituximab and could potentially have therapeutic repercussions. The induction of polyreactivity could represent a new axis for improvement of the therapeutic potential of Rituximab. The project is funded by ERC (StG-2015 CoBABATI) and Association for Cancer Research (ARC, France) grants.

WS.B1.02.06

Evaluation of a New Anti-Galectin 9 Immunotherapy Strategy in Pancreatic Cancers

A. Quilbe[1], *R. Mustapha*[1], *S. Renaud*[1], *B. Duchêne*[2], *C. De Schutter*[1], *G. Herlin*[1], *O. Moralès*[1], *I. Van Seuningen*[2], *N. Jonckheere*[2], *N. Delhem*[1];
[1]*CNRS UMR 8161, Institut de Biologie de Lille, 59021 Lille Cedex, France,* [2]*INSERM UMR837 Team 5, 59021 Lille Cedex, France.*

Background: We have previously described in an humanized mouse model of nasopharyngeal carcinoma that an anti-Galectin-9 (Gal-9) monoclonal antibody (mAb) is able to significantly limits tumor growth by specifically inhibiting the suppressive activity of human natural Tregs (Patent WO: WO2015185875). Herein, we propose to use this new specific active immunotherapy in a pancreatic cancer mouse model ($KRAS^{G12D}$), insofar as a high Treg prevalence has been described and correlated to the tumor progression of pancreatic cancer.
Methods and Results: Gal-9 expression was confirmed by immunohistochemistry on pancreas isolated from the $KRAS^{G12D}$ mouse model. This Gal-9 expression level has been correlated to the progression of pre-cancerous lesions. Furthermore, an increase of Tregs prevalence has been observed in this transgenic model at a systemic and intratumoral level. Very interestingly, we also showed that **(i)** murine Tregs expressed Gal-9 (flow cytometry, immunofluorescence and western-blot), **(ii)** anti-Gal9 mAb neutralized the immunosuppression induced by recombinant murine Gal-9 (proliferation assays) and **(iii)** anti-Gal9 mAb neutralized the suppressive activity of murine Tregs (MLR assay). Further investigations performed on four human pancreatic cancer cell lines (Capan-1, Capan-2, MIAPaCa-2, Panc-1) have also confirmed the Gal-9 expression at a genomic (RT-qPCR), proteomic (Immunofluorescence, Western-blot and flow cytometry) and secreting (ELISA) level.
Conclusion: Our preliminary results suggest that the use of an anti-Gal-9 mAb could be considered as a new anti-tumoral immunotherapy targeting Tregs in the pancreatic cancer.

WS.B1.03 Genetically engineered TCR for immunotherapy

WS.B1.03.01

Development and characterization of novel anti-GD2 target modules for retargeting of Universal CAR T cells toward GD2 expressing tumors

*N. Mitwasi[1], **A. Feldmann**[1], R. Bergmann[1], N. Berndt[1], C. Rössig[2], M. Bachmann[1,3,4];*
[1]Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Dresden, Germany, [2]Department of Pediatric Hematology and Oncology, Münster University Hospital (UKM), Dresden, Germany, [3]Tumor Immunology, University Cancer Center (UCC) 'Carl Gustav Carus' TU Dresden, Dresden, Germany, [4]German Cancer Research Center (DKFZ), Heidelberg, Germany.

Although chimeric antigen receptor (CAR) engineered T cells demonstrated promising therapeutic effect against cancer, they are still associated with adverse side effects which could be life threatening in some cases. Therefore, in our group we have developed a switchable universal CAR T cell platform "UniCAR", which can be repeatedly switched on and off. This system consists of CAR T cells that cannot bind tumor antigens directly but instead they are redirected with a target module (TM). Such TMs are mainly composed of an epitope on one side, which is recognized by the UniCAR T cells, and on the other side a tumor antigen-binding domain. Once the TM is eliminated, the UniCAR T cells are no more activated. Disialoganglioside GD2 was shown previously to be a very promising target for several tumors such as neuroblastoma and Ewing's sarcomas. Therefore, anti-GD2 TMs were developed and evaluated regarding their functionality. They were shown to be functional in activating the UniCARs to secrete important pro-inflammatory cytokines and to kill $GD2^+$ tumor cells both in vivo and in vitro. To further characterize the anti-GD2 TM with PET imaging, it was labeled with radioactive Cu^{64}. The TM showed a specific enrichment at the site of the $GD2^+$ growing tumor, and it was mainly eliminated through the kidneys within half an hour due to its small size. Such short half-life, provide the UniCAR system with the fast safety switch in case any complications occurred in patients treated with the UniCAR T cells.

WS.B1.03.02

Functional comparison of CARs targeting CD20 with a TCR directed against a CD20 derived peptide

***T. L. A. Wachsmann**[1], L. Jahn[1], E. van Diest[2], J. Leusen[2], J. Kuball[2], J. Falkenburg[1], M. Heemskerk[1];*
[1]LUMC, Leiden, Netherlands, [2]UMC Utrecht, Utrecht, Netherlands.

With the rise of chimeric antigen receptor (CAR) T-cells, the role & potential of T-cell receptors (TCRs) targeting surface antigen derived peptides for gene transfer therapy needs to be reevaluated. While the non HLA-restricted CAR T-cells have demonstrated remarkable clinical efficacy in hematological malignancies, severe toxicity in responders and non-responsiveness in a subset of patients remain major challenges. We hypothesize that TCR transduced T-cells targeting tumor surface antigen derived peptides pose a valuable alternative to CAR T-cells by maintaining a comparable efficacy while exhibiting a more tolerable toxicity profile, alongside higher resistance to activation induced cell death and exhaustion.
Our group has previously identified a high affinity TCR targeting a CD20 derived peptide presented in the context of HLA-A2. Using a panel of human acute lymphoblastic leukemia cells with different levels of CD20 expression, we aim to functionally compare our TCR with four 4-1BB-CD3z second generation CARs differing in their CD20 recognition domain. Primary endpoints are functionality (killing capacity & cytokine profiling), resilience, proliferative capacity and maintenance of functionality after antigen challenge.
Preliminary *in vitro* studies reveal markedly elevated cytokine production alongside indications of accelerated killing kinetics in the CAR T-cells as opposed to the TCR transduced T-cells in a CD20 expression sensitive manner. However, this appears to trade off against activation induced cell death and severe impairment of proliferative capacity in response to high levels of antigen exposure in the CAR T-cells. Ultimately, our experimental framework provides a rationale for the future direction of antigen receptor design.

WS.B1.03.03

Impaired Early Downstream Signaling Blunts Antigen Sensitivity of CAR-T-cells

***V. Gudipati**[1], J. Rydzek[2], I. Perez[1], S. Königsberger[1], H. Stockinger[1], M. Hudecek[2], J. Huppa[1];*
[1]Medical University of Vienna, Vienna, Austria, [2]Universitätsklinikum Würzburg, Würzburg, Germany.

Adoptive immunotherapy employing chimeric antigen receptor (CAR)-modified T-cellshas given rise to new hope in oncology as an effective treatment regimen for advanced malignancies. While high rates of complete remission after CAR T-cell therapy can be obtained in patients with B cell malignancies, relapse may occur in significant number of patients, often owing to antigen loss variants. Rational design of CARs with optimized anti-cancer performance mandates detailed knowledge of how CARs engage tumour antigens and how antigen-engagement triggers activation. To gain a deeper insight into the mechanisms of CAR-induced activation and the development of the CAR immunological synapse, we employed total internal reflection fluorescence (TIRF) microscopy. We found the sensitivity of CAR-T-cells towards antigen is reduced by 500 times when compared to T-cell antigen receptor-mediated detection of nominal peptide/MHC complexes. While CAR-antigen binding was efficient, receptor-proximal signalling was significantly attenuated due to reduced recruitment of the tyrosine kinase ZAP70 at ligated CARs. At limiting antigen densities absence of adhesion molecule ICAM1 significantly affects CAR T-cell mediated cytotoxicity indicating that blunted CAR signalling leads to attenuated activation of the integrin LFA-1, thereby compromising cell adhesion. our findings expose fundamental limitations of current one-dimensional CAR designs that has to be overcome for personalized cancer treatment. Furthermore, our findings highlight unique strengths of live molecular imaging for preclinical CAR-development.

WS.B1.03.04

Antitumor activity by TEGs: alpha/beta T cells engineered to express a defined gamma/delta TCR in a 3D bone marrow niche model of multiple myeloma

***T. Straetemans**[1], M. Braham[2], T. Aarts-Riemens[1], J. Alblas[3], M. Minnema[4], Z. Sebestyen[1], J. Kuball[4];*
[1]Laboratory of Translational Immunology, University Medical Center Utrecht, Utrecht, Netherlands, [2]Department of Orthopaedics, University Medical Center Utrecht, Utrecht, Netherlands, [3]Department of Orthopeadics, University Medical Center Utrecht, Utrecht, Netherlands, [4]Department of Hematology, University Medical Center Utrecht, Utrecht, Netherlands.

γδT cells mediate cancer immune surveillance by sensing metabolic changes of malignant cells via their γδTCR. This concept led to the development of next generation CAR T cells, so-called TEGs: αβT cells Engineered to express a defined γδTCR. A particular γ9δ2TCR, has been selected as candidate for clinical testing (TEG001). TEG001 cells showed a strong and broad recognition of hematological malignancies and are able to differentiate between healthy and leukemic stem cells. Important for the therapeutic success of immune therapy concepts such as TEGs is a better understanding of the interplay between malignant, stromal and immune cells in the tumor microenvironment. To this end a 3D model was established that allowed engraftment of primary multiple myeloma (MM) cells within a humanized bone marrow niche...TEG001 cells were engineered from both healthy donor and MM patients' T cells and added when MM growth was established. TEG001 cells, but not mock engineered T cells, migrated into the 3D structure and exerted a killing response towards the tumor cells but not towards the stromal cells. This cognate recognition was associated with the differential production of chemokines, cytokines and inhibitory molecules. Amongst others, TEG001 cells induced CCL1 secretion, but also the secretion of IL-6 and GM-CSF, reported to be involved in cytokine release syndrome. Soluble Galectin-9, the proposed ligand for inhibitory receptor TIM-3, was reduced in the supernatant. These findings demonstrate that TEGs are a promising addition to the currently available immune therapeutic strategies as they target cancer as a metabolic disease.

WS.B1.03.05

Engineering antigen-specific Natural Killer cells against the melanoma-associated antigen tyrosinase via TCR gene transfer

***A. Parlar**[1,2], C. Pamukcu[1,2], E. C. Sayitoglu[3], A. Georgoudaki[3], D. Ozkazanc[1,2], M. Aras[1,2], M. Chrobok[3,4], P. Zahedimaram[1,2], L. Ikromzoda[1,2], E. Alici[3,4], B. Erman[1,2], A. D. Duru[3], T. Sutlu[1];*
[1]Nanotechnology Research and Application Center, Sabancı University, Istanbul, Turkey, [2]Faculty of Engineering and Natural Sciences, Sabancı University, Istanbul, Turkey, [3]NSU Cell Therapy Institute, Nova Southeastern University, Florida, United States, [4]Center for Hematology and Regenerative Medicine, Karolinska Institutet, Stockholm, Sweden.

While genetic modification of cytotoxic T lymphocytes (CTLs) using T Cell Receptors (TCRs) to target intracellular antigens or using Chimeric Antigen Receptors (CARs) to target cell surface antigens are commonly investigated; for Natural Killer (NK) cells, CARs have so far been the only practical method of antigen-specific retargeting, TCR gene therapy can supply large populations of CTLs genetically modified to express a specific TCR, but the mispairing of endogenous and genetically transferred TCR subunits constitutes a bottleneck in the development of safe therapies. In order to overcome this obstacle and open the realm of intracellular antigens to targeting by NK cells, we propose to use NK cells for TCR gene therapy. Our results show that ectopic expression of the CD3δ, CD3γ, and CD3ε chains along with TCR α/β gene delivery to NK cells enables the functional expression of a TCR specific to tyrosinase-derived peptide $Tyr_{368-379}$ in complex with HLA-A2. We observed that neither the TCR α/β heterodimer, nor the CD3 subunits had the capacity to transport to the cell surface alone but could only form a stable complex when all components were present. The introduction of a functional TCR complex to NK cells enabled antigen-specific and MHC-restricted triggering of effector functions against tyrosinase expressing tumor cells both *in vitro* and *in vivo*. This strategy not only opens the realm of intracellular antigens to targeting by NK cells but also provides a definite solution for the mispairing problem observed in TCR gene therapy.

WS.B1.03.06

TCR replacement in the endogenous locus via non-viral CRISPR-Cas9-mediated knock-in

K. Schober[1], *T. Müller*[1,2], *D. H. Busch*[1,2,3];
[1]*Institute of Medical Microbiology, Immunology and Hygiene, München, Germany,* [2]*National Center for Infection Research (DZIF), Munich, Germany,* [3]*Focus Group "Clinical Cell Processing and Purification", Institute for Advanced Study, TUM, Munich, Germany.*

Recently emerged advanced genome editing tools like CRISPR/Cas9 open up new avenues for the development of next-generation cell therapies, including T cell therapy for the treatment of viral infections or cancer. Knockout of the endogenous T cell receptor (TCR) has been reported to both increase the functionality of TCR-transduced T cells and reduce the risk of Graft-versus-Host-Disease (GvHD) (Provasi et al. Nat Med 2012). Adeno-associated virus (AAV)-mediated delivery of a chimeric antigen receptor (CAR) into the CRISPR/Cas9-edited TCR locus has been achieved with high editing efficiency and revealed superior *in vivo* functionality due to physiological receptor expression regulation (Eyquem et al. Nature 2017). Recently, non-viral CRISPR/Cas9-mediated TCR replacement in the endogenous locus has been realized (Roth et al. bioRxiv 2017), but a systematic investigation of the contribution of 'lacking competition' through the endogenous TCR on the one side, and physiological transgenic TCR expression on the other side, has not been undertaken. Combining CRISPR/Cas9-mediated TCR replacement with a library of differential avidity TCRs directed against the same epitope (e.g. A2-pp65), we have set up a platform to in-depth dissect the parameters leading to superior functionality of 'replacing TCRs'. Simultaneously, we show that through-put of TCR characterization - so far the key bottleneck in TCR testing platforms - can be massively accelerated by CRISPR/Cas9-mediated re-expression. In consequence, TCR candidates for engineered T cell therapeutics can not only be assessed more quickly, but also in their physiological regulatory framework.

WS.B1.04 Antigen specificity in anti-tumor immunity

WS.B1.04.01

Towards the next generation CAR T cells with TEGs: in vivo efficacy - toxicity profile in xenografts of primary human AML disease and healthy bone marrow

I. Johanna[1], *T. Straetemans*[1], *S. Heijhuurs*[1], *T. Aarts-Riemens*[1], *H. Norell*[2], *L. Bongiovanni*[3], *A. de Bruin*[3], *Z. Sebestyen*[1], *J. Kuball*[1];
[1]*Department of Hematology and Laboratory of Translational Immunology, University Medical Center Utrecht, Utrecht, Netherlands,* [2]*Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal,* [3]*Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands.*

γδT cells mediate cancer immune surveillance by sensing metabolic changes of malignant leukemic blasts and not their healthy counterpart via their γδT cell receptor (TCR). This concept led to the development of next generation CAR T cells, so-called TEGs: αβT cells Engineered to express a defined γδTCR. A particular γ9δ2TCR, isolated from "clone 5", has been selected as the candidate for clinical testing (TEG001). TEG001 cells showed a strong and broad recognition of hematological malignancies against both cell lines and primary AML.
In order to evaluate the biodistribution and safety profile of TEGs we developed a patient-derived xenograft (PD-X) *in vivo* model by establishing primary malignant AML blasts, which tested positive in an *in vitro* assay for recognition by TEGs, in NSG mice. In addition, healthy stem cells from human cord-blood were in parallel engrafted in a separate set of NSG mice. After engraftment, TEGs were infused and mice followed for additional 50 days. While engrafted primary AML blasts were no longer detectable in the peripheral blood at the end of the study period, all healthy hematological cellular compartments remained unharmed.
Within the limitations of humanized PD-X models, TEGs target acute myeloid leukemia but do neither interfere with engraftment of hematopoietic progenitors nor harm matured subsets of the hematopoiesis. In addition, no additional signs of off-target toxicity were observed in mice. TEGs are a promising addition to the currently available immune therapeutic strategies as they target cancer as a metabolic disorder.

WS.B1.04.02

Harnessing Pre-Existing Antiviral Immunity to Treat Solid Tumors

N. Cuburu[1], *R. Kim*[1], *S. M. Pontejo*[2], *C. D. Thompson*[1], *D. R. Lowy*[1], *J. T. Schiller*[1];
[1]*National Cancer Institute, NIH, Bethesda, United States,* [2]*National Institute of Allergy and Infectious Diseases, NIH, Bethesda, United States.*

Human cytomegalovirus is highly prevalent in humans with polyfunctional T cell responses expanding with age. We questioned whether redirecting pre-existing anti-cytomegalovirus T cells into solid tumor could arrest tumor growth, induce epitope spreading, and confer long-term anti-tumor immunity.Persistently infected mice with murine cytomegalovirus (mCMV) were challenged with TC-1 tumor cells expressing human papillomavirus (HPV) E6 and E7 oncogenes. In vivo transduction of TC-1 tumors with a viral vector expressing MCMV antigens or intratumoral injection of peptidic mCMV epitopes with a TLR3 agonist (poly I:C) caused the expansion of mCMV-specific CD4+ and CD8+ T cells. Using PanCancer Imune profiling panel (Nanostring) we show that intratumoral injection of mCMV peptide epitopes induced massive modifications of the tumor innate and adaptive immune environment. Intratumotal injection of mCMV CD8 peptide epitopes provoked the arrest of tumor growth. Intratumotal injection of mCMV CD4 peptide epitopes with poly I:C promoted induction of E7-specific CD8+ T cells. Sequential administration of CD4 and CD8 mCMV epitopes together with poly I:C was the best protocol to eradicate pre-existing tumor of 5 to 10mm diameter, and rechallenge experiments showed antitumor immunity up to 4 months after the last treatment.Our results provide a proof of concept to design "antigen-agnostic" intratumoral therapies based on pre-existing antiviral T cells. Such approach changedthe tumor immune microenvironment, induced epitope spreading, and conferred long term anti-tumor immunity. These findings prompt further evaluation in other spontaneous tumor models and provide a model to decipher the mechanisms of epitope spreading notably to investigate CD4 T cell help.

WS.B1.04.03

Ephemeral immune control of cytomegalovirus infection by T-cells recognizing a single viral epitope

F. Mbui, *L. Borkner, Z. Chaudhry, L. Cicin-Sain;*
Helmholtz Center for Infection Research (HZI), Braunschweig, Germany.

The cytotoxic T cells play an important role in the control of cytomegalovirus (CMV). T cell-based adoptive immunotherapy using antigen specific cells is explored as a treatment option for CMV disease. However, therapeutic success varies among individual recipients and the underlying reasons remain unclear and unpredictable. To understand the minimal requirements for adoptive immunotherapy of CMV disease, we generated recombinant murine CMVs expressing the immunodominant epitopes SSIEFARL or KCSRNRQYL. We infected TCR transgenic mice on a $RAG2^{-/-}$ background, recognizing only these epitopes, and followed their survival upon infection. While wild-type virus rapidly killed the mice, recombinant viruses expressing the corresponding epitope were controlled. However, the immune protection was transient, because the mice succumbed by 6-8 weeks post infection. To test if poor viral control was caused by T-cell exhaustion, we analyzed their phenotype. While we observed an accrual of exhausted T cells ($PD1^+$, $Eomes^+$) at times of death, these increases were modest. Most T cells remained PD1-negative and retained functionality in ex vivo assays. Alternatively, viral escape of immune recognition could have explained the phenomenon. Therefore, we isolated MCMV genomes from organs of infected mice at time of death and sequenced them. We identified epitope deleterious mutations in the vast majority of viral genomes, which were sufficient to prevent T-cell recognition of infected cells. Our data argue that T-cell immunotherapy against one immunodominant epitope may provide transient protection against CMV, but also drive immune escape. Therefore, our data indicate that optimal T-cell protection may require targeting multiple epitopes.

WS.B1.04.04

Autologous neo-antigen-specific T cell responses in low mutation burden colorectal cancers

J. van den Bulk, *D. Ruano, M. Visser, M. IJsselsteijn, R. van der Breggen, K. Peeters, S. van der Burg, E. Verdegaal, N. de Miranda;*
LUMC, Leiden, Netherlands.

Innovative treatment options are required to improve cure rates in advanced colorectal cancer patients. Immune checkpoint blockade therapy (anti-PD-1) was shown to be effective in colorectal cancers with high mutation burden (e.g. mismatch repair deficient) as anti-tumour reactivity is largely explained by the recognition of somatically mutated antigens (neo-antigens). No immunotherapeutic strategies are currently available for patients diagnosed with low mutation burden CRC, while they could greatly benefit from the induction of immune responses. We hypothesized that if autologous neo-antigen-reactive T cells are present in such patients, they might benefit from specific immunotherapeutic interventions that stimulate neo-antigen recognition. In order to detect neo-antigens, whole exome and RNA next-generation sequencing were performed in cancer and healthy tissues from colorectal cancer patients. Corresponding peptides were synthesized and tested for their ability to induce immune cell activation in lymphocytes isolated from the tumour tissue and from peripheral blood. Neo-antigen-specific T cell responses were identified against 5 out of 39 neo-antigens corresponding to 35 somatic mutations that were expressed in the tumour tissue from a CRC patient. In conclusion, we developed a neo-antigen screening pipeline to unlock the immunogenic potential of colorectal cancers with low mutation burden. We have detected a relatively high number of neo-antigens that are recognized by autologous T cells in a mismatch repair proficient, low mutation burden CRC patient. This finding supports the widespread evaluation of the potential to employ neo-antigen-targeted therapies to improve the treatment of colorectal cancer patients.

WS.B1.04.05

A minority of tumor associated antigen specific T-cells restricted to self-HLA alleles is of sufficient avidity to recognize overexpressed endogenously processed antigen

M. Roex[1], *L. Hageman*[1], *E. van Egmond*[1], *S. Veld*[1], *C. Hoogstraten*[1], *L. Germeroth*[2], *F. Falkenburg*[1], *I. Jedema*[1];
[1]*Leiden University Medical Center, Leiden, Netherlands,* [2]*Juno Therapeutics, Göttingen, Germany.*

Tumor associated antigens (TAA) are proposed as targets for graft versus leukemia effect after HLA-matched allogeneic stem cell transplantation. As TAA are self-antigens, high avidity TAA-specific T-cells are thought to be eliminated from the T-cell repertoire by thymic selection. In this study, we investigated whether TAA-specific T-cells with sufficient avidity to recognize overexpressed endogenously processed antigen in self-HLA can be found in healthy donors. T-cells directed against TAA peptides NY-eso-1-SLL/A*02:01, WT1-RMF/A*02:01, RHAMM-ILS/A*02:01, Proteinase-3-VLQ/A*02:01 and PRAME-VLD/A*02:01 were isolated from HLA-A*02:01+ donors using MHC-I-*Strep*tamers. Generated tetramer+/CD8+ T-cell clones were classified based on the minimal concentration of peptide exogenously loaded on TAP-deficient T2-cells needed for cytokine production. To analyze recognition of overexpressed endogenously processed antigen, high-potential clones were tested against HLA-A*02:01+ EBV-LCL transduced with the full corresponding TAA sequence. >800 tetramer+/CD8+ TAA-specific clones were isolated from 18 donors. T-cell receptor Vbeta-family analysis revealed a minimal number of unique clones: 14 NY-eso-1-SLL/A*02:01, 30 WT1-RMF/A*02:01, 14 RHAMM-ILS/A*02:01, 8 Proteinase-3-VLQ/A*02:01 and 14 PRAME-VLD/A*02:01 clones. The functional screening revealed 27 non-functional clones, 16 low-potential clones (activation threshold ≥10^-6M peptide) and 37 high-potential clones (activation threshold ≤10^-7M peptide). Of the high-potential clones, only 2 NY-eso-1-SLL/A*02:01, 7 WT1-RMF/A*02:01 and 5 PRAME-VLD/A*02:01 clones showed recognition of overexpressed endogenously processed antigen. These results illustrate that self-HLA restricted TAA-specific T-cells can be easily isolated from donor PBMC, but that only a minority of T-cells are capable of recognizing overexpressed endogenously processed antigen. Classification of functional TAA-specific T-cells by only high tetramer staining and peptide specificity leads to overestimation of relevant avidity.

WS.B1.04.06

Cross-reactive CD8+ T cell receptor clonotypes elicited by influenza epitope variants are drawn from a narrow repertoire distinct from non-cross-reactive receptors

P. G. Thomas*, P. Dash, P. Bradley, A. Williams, S. Duan;*
St. Jude Children's Research Hospital, Memphis, United States.

T cells specific for one peptide-MHC (pMHC) epitope can occasionally cross-react with closely related pMHC, such as those containing single amino acid mutations. What determines the extent of cross-reactivity has not been well-characterized. To define the "rules" of cross-reactivity, we chose two variants of the influenza D^bNP_{366}(ASNENMETM, NP-wt) epitope with a single residue change, (NP-T8A) and (NP-N3A), that are known to elicit cross-reactive responses to NP-wt. We analyzed the paired αβ TCR repertoire of cells recognizing these epitopes from mice infected with each of the variant viruses singly or in combination in a prime-challenge model. Each combination elicited highly variable degrees of cross-reactivity, ranging from 20% (T8A elicited cells reacting to wt epitope) to 90% (N3A-elicited cells reacting to wt epitope). Most strikingly, regardless of the magnitude of cross-reactivity, cross-reactive cells between each NP variant displayed an extremely narrow TCR repertoire diversity, often dominated by a single clonotype, while the non-cross-reactive cells had markedly higher diversity in their TCR repertoires. Importantly, many of these cross-reactive receptors did not appear to be the dominant receptors responding to the eliciting epitope (i.e. they had a higher TCRdistance). Our results indicate that cross-reactivity is mediated by a rare subset of TCRs that are not representative of the typical responses elicited by an epitope. This work has implications for tumor immunity, as it provides insight into how to avoid self-reactivity, and vaccine design, as repeated immunizations with distinct epitopes are likely to select for extremely narrow, cross-reactive repertoires.

WS.B1.05 Anti-tumor immunology

WS.B1.05.01

dissecting the immune heterogeneity of neuroblastoma microenviroment in murine models to develop novel therapeutic strategies for high-risk neuroblastoma patients

V. Lucarini*, O. Melaiu, M. Vinci, M. Compagnone, G. Zicchedda, L. Cifaldi, V. Pistoia, D. Fruci;*
Ospedale Pediatrico Bambino Gesù, Rome, Italy.

Introduction The presence of tumor-infiltrating T cells (TILs) and the absence of immunosuppressive elements have been associated with favourable prognosis of high-risk neuroblastoma (NB) patients. Recently, a subset of intratumoral dendritic cells (iDC) has been found crucial for anthracycline-induced anticancer immune responses suggesting that they might be exploited to improve NB therapy. Herein, we investigated the modulation of the immune infiltrate in NB murine models treated with chemotherapeutic drugs in combination with immune checkpoint blocking antibodies. **Methods** Luciferase-expressing NB cell lines derived from spontaneous tumors arising in the TH-MYCN transgenic mice were injected subcutaneously or orthotopically in syngeneic mice. Mice bearing established tumors were sacrificed and TILs were analysed by flow cytometry. The crosstalk between immune and tumor cells was evaluated in drugs-treated NB spheroids co-cultured with tumor-infiltrating $CD45^+$ cells. **Results** Tumor microenviroment was characterized by a consistent number of $CD45^+$ immune cells, including T cells, NK cells, NKT cells, macrophages, neutrophils and iDCs. The orthotopic model showed a more aggressive phenotype than the subcutaneous model, resulting in the development of tumors with an immunosuppressive microenvironment predominantly infiltrated by macrophages, myeloid-derived suppressor cells and T regulatory cells. Treatment with anthracycline-derived drugs resulted in a reduced diameter of spheroid and an increased recruitment of TILs. *In vivo* experiments are currently underway to evaluate the TILs in tumor-bearing mice treated with anthracycline-derived drugs and immune checkpoint blocking antibodies. Overall, we provide insights for the study of a novel immunotherapeutic approach in NB.

WS.B1.05.02

Deficiency of host CD96 and PD-1 or TIGIT enhances tumor immunity without significantly compromising immune homeostasis

H. Harjunpaa[1,2], *S. J. Blake*[1], *E. Ahern*[1,2,3], *S. Allen*[1], *J. Liu*[1], *J. Yan*[1,2], *V. Lutzky*[1], *K. Takeda*[4], *A. Roman Aguilera*[1], *C. Guillerey*[1,2], *D. Mittal*[1,2], *X. Y. Li*[1], *W. C. Dougall*[1], *M. J. Smyth*[1,2], *M. W. Teng*[1,2];
[1]*QIMR Berghofer Medical Research Institute, Brisbane, Australia,* [2]*The University of Queensland, Brisbane, Australia,* [3]*Royal Brisbane and Women's Hospital, Brisbane, Australia,* [4]*Juntendo University, Tokyo, Japan.*

Multiple non-redundant immunosuppressive pathways co-exist in the tumor microenvironment and their co-targeting can increase clinical responses. Indeed, concurrent blockade of CTLA-4 and PD-1 in patients with advanced melanoma increased clinical responses over monotherapy although the frequency and severity of immune related adverse events also increased. Nevertheless, a substantial number of patients still display an innate resistance phenotype and are unresponsive to current approved immunotherapies even when utilized in combination. In this study, we generated *Pdcd1*$^{-/-}$*CD96*$^{-/-}$ and *Tigit*$^{-/-}$*CD96*$^{-/-}$ mice to investigate how loss of CD96 in combination with PD-1 or TIGIT impacts on immune homeostasis and hence the potential of inducing immune related toxicities following co-targeting of these pairs of receptors. The ability of *Pdcd1*$^{-/-}$*CD96*$^{-/-}$ and *Tigit*$^{-/-}$*CD96*$^{-/-}$ mice to suppress primary tumor growth was also assessed using the MC38 colon carcinoma and SM1WT1 BRAF-mutated melanoma tumor models. Both *Pdcd1*$^{-/-}$*CD96*$^{-/-}$ or *Tigit*$^{-/-}$*CD96*$^{-/-}$ mice displayed no overt perturbations in immune homeostasis over what was previously reported with *Pdcd1*$^{-/-}$ or *Tigit*$^{-/-}$ mice even when aged for 22 months. Interestingly, increased suppression of subcutaneous tumor growth and complete responses was seen in *Pdcd1*$^{-/-}$*CD96*$^{-/-}$ mice compared to *Pdcd1*$^{-/-}$ or *CD96*$^{-/-}$ mice depending upon the tumor model. This enhanced anti-tumor efficacy of *Pdcd1*$^{-/-}$*CD96*$^{-/-}$ mice appeared to be due to favorable changes in the ratio of $CD8^+$T cells to Tregs or $CD11b^+GR\text{-}1^{hi}$ myeloid cells in the tumor microenvironment. Co-targeting CD96 and PD-1 may increase anti-tumor immunity over targeting PD-1 alone and potentially not induce serious immune-related toxicities and thus appears a promising strategy for clinical development.

WS.B1.05.03

Stromal Protein βig-h3 Reprograms Tumor Microenvironment in Pancreatic Cancer

D. Goehrig[1], *J. Nigri*[2], *R. Samain*[3], *Z. Wu*[4], *C. Paola*[5], *G. Gabiane*[4], *X. Zhang*[4], *Y. Zhao*[4], *I. Kim*[6], *M. Chanal*[4], *R. Curto*[5], *V. Hervieu*[7], *C. de la Fouchardière*[8], *F. Novelli*[5], *P. Milani*[9], *R. Tomasini*[2], *C. Bousquet*[3], *P. Bertolino*[10], ***A. HENNINO***[10];
[1]*INSERM 1052, Lyon, France,* [2]*INSERM 1068, Marseille, France,* [3]*INSERM 1037, Toulouse, France,* [4]*INSERM 1052, LYON, France,* [5]*University of Turin, Turin, Italy,* [6]*KIST, Seoul, Korea, Republic of,* [7]*Hospices Civiles Lyon, LYON, France,* [8]*Centre Léon Bérard, LYON, France,* [9]*ENS Lyon, LYON, France,* [10]*CRCL, INSERM 1052, Lyon, France.*

Pancreatic cancer is associated with an abundant stromal reaction leading to immune escape and tumor growth. This massive stroma drives the immune escape in the tumor. We identified βig-h3 stromal protein as a key actor of the immune paracrine interactions mechanism that drives pancreatic cancer. We performed studies with *p48*-Cre;*Kras*^{G12D}, *pdx1*-Cre;*Kras*^{G12D};*Ink4a/Arf*$^{fl/fl}$, *pdx1*-Cre;*Kras*^{G12D}; *p53*^{R172H} mice and tumor tissues from patients with PDA. Some transgenic mice were given injections of anti-βig-h3 depleting antibody (Ab). Tumor growth as well as modifications in the activation of local immune cells were analyzed by flow cytometry, immunohistochemistry, immunofluorescence and stiffness by atomic force microscopy.
We found that βig-h3 is highly produced by Cancer Associated Fibroblasts in the stroma of Human and mouse. This protein acts directly on tumor specific-$CD8^+$ T cells and F4/80 macrophages. Depleting βig-h3 *in vivo* reduced tumor growth by enhancing the number of activated $CD8^+$ T cell within the tumor and subsequent apoptotic tumor cells. More importantly, we found that targeting βig-h3 in established lesions increased immune-mediated tumor clearance by releasing the tissue tension and functionally reprogramming F4/80 macrophages in the tumor microenvironment. Our findings present βig-h3 as a novel immunological target in pancreatic cancer.

WS.B1.05.04

Retargeting T cells against leukemia by lipid-specific TCR transfer

M. Consonni[1], *C. Garavaglia*[1], *C. de Lalla*[1], *A. Bigi*[1], *A. Mancino*[1], *M. Casucci*[2], *A. Bondanza*[2], *M. Lepore*[3], *L. Mori*[3], *G. De Libero*[3], *F. Ciceri*[4], *P. Dellabona*[1], *G. Casorati*[1];
[1]San Raffaele Scientific Institute, Division of Immunology, Transplantation, and Infectious Diseases, Experimental Immunology Unit, Milano, Italy, [2]San Raffaele Scientific Institute, Division of Immunology, Transplantation, and Infectious Diseases, Innovative immunotherapies Unit, Milano, Italy, [3]Experimental Immunology, Department of Biomedicine, University Hospital, Basel, Switzerland, [4]San Raffaele Scientific Institute, Division of Regenerative medicine, Stem cells, and Gene therapy, Hematology and hematopoietic stem cell transplantation Unit, Milano, Italy.

CD1-restricted T cells specific for self-lipids could play a role in cancer immune surveillance, where self-antigens are the targets of immune responses. We showed that primary acute myelogenous (AML) and B-lymphoblastic (B-ALL) leukemia blasts express CD1c and are recognized by a group of CD1c self-reactive T cells specific for methyl-lysophosphatidic acids (mLPAs), a novel class of self-lipid antigens that accumulate in malignantcells. mLPA specific T cells can kill and control leukemia growth *in vitro*and *in vivo*. These findings point to CD1c and self-lipids as new potential targets for leukemia immunotherapy. The little polymorphism of CD1 molecules and their selective expression on mature leukocytes are indeed highly attractive for adoptive cell therapy (ACT) with such T cells in the context of stem cell transplantation for hematological malignancies. To assess the feasibility of ACT for acute leukemia with mLPA-specific T cells, we generated a library of lentiviral vectors encoding a panel of human mLPA-specific TCRs. Upon TCR transduction, either Jurkat T cells or human primary T cells were specifically retargeted against CD1c-expressing malignant targets *in vitro*, highlighting a lead mLPA-specific TCR suitable for adoptive immunotherapy. Primary T cells transduced with this TCR killed CD1c-expressing malignant targets *in vitro* and significantly delayed leukemia progression in NSG mice. Togain further insight into the efficacy and safety of mLPA-specific ACT, we generated transgenic mice expressing CD1cwith a pattern similar to the human one, which harbored functional APCs recognized by mLPA-specific T cells and selected a CD1c self-reactive peripheral T cell repertoire.

WS.B1.05.05

Chorioallantoic membrane model to study glycan dependent tumor growth and differentiation

N. M. Sahasrabudhe*, L. Cornelissen, K. Castricum, J. van der Horst, V. L. Thijssen, S. J. van Vliet;
VU University medical center, Amsterdam, Netherlands.*

Immune activation in the colon plays a major role in colorectal cancer (CRC) progression, as exemplified by the many cases of CRC that develop from inflammatory bowel diseases. We have determined that glycans present on the colorectal tumors are vital epitopes that lead to immune evasion mechanisms in late stage tumors. Macrophages are the main tumor-associated immune cells, wherein the activation status of tumor-associated macrophages (TAM) is dependent on the tumor microenvironment. We hypothesize that glycans in tumor microenvironment influence macrophage differentiation. To decipher the role of glycans in CRC, we developed mouse MC38 CRC cell line expressing tumor associated glycans using CRISPR-Cas9 genome editing. We have knocked out *COSMC* gene to increase Tn antigen (GalNAcα-1Ser/Thr) exposure. To study growth of these glycovariant tumors we used the chorioallantoic membrane (CAM) assay with fertilized chicken eggs as a model. The CAM acts as the growth surface for development of solid tumors using cancer cell lines. This method allowed us to study tumor growth characteristics in a physiological 3D model along with angiogenesis and differentiation potential of the tumor. We observed higher angiogenesis potential of the MC38 *COSMC* knockout cell lines, suggesting a role for Tn antigen. In addition, we have modified the CAM assay to include monocytes in the tumor to study glycan-dependent monocyte differentiation into TAMs. Our objective is to elucidate the specific glycan epitopes that lead to immune evasion mechanisms in tumors through immunoregulatory macrophages.

WS.B1.05.06

An NK cell-driven inflammatory switch regulated by cyclooxygenase-2 activity restricts tumor growth and predicts cancer patient outcome

E. Bonavita*, V. S. Pelly, C. Bromley, E. Flanagan, S. Sahoo, H. Leong, S. Zelenay;
CRUK Manchester Institute, Manchester, United Kingdom.*

Inflammation plays opposing roles in cancer. It can either support or restrain tumor growth depending on the composition and levels of various cellular and molecular mediators. Nevertheless, how different types of inflammatory environments are established and manipulated by a growing tumor remains unclear. Studying cancer models rendered immunogenic by tumor cell-specific ablation of the cyclooxygenase (COX)-2 pathway, we showed the necessity of natural killer (NK) cells in establishing a cancer-inhibitory microenvironment that precedes activation of conventional dendritic cell 1 (cDC1) and drives cytotoxic T cell (CTL)-dependent tumor control. NK cells were found to be equally essential for the efficacy of immunotherapy based on combinations of a COX-2 inhibitor and PD-1 blockade. In agreement, *in silico* analysis of cancer patient datasets suggested that the COX-2 pathway similarly regulates the cellular and molecular inflammatory profile across multiple human malignancies. A gene signature that combines COX-2-dependent tumor-promoting factors and NK cell-driven anti-tumor mediators discriminated cancer types with diverging immune cell composition and predicted overall patient survival and response to PD-1/PD-L1 blockade. Collectively our findings demonstrate a major role for the COX-2 pathway in determining the inflammatory nature of tumors and that monitoring its driven gene signature constitutes a powerful biomarker of cancer patient outcome.

WS.B1.06 Anti-tumor strategies

WS.B1.06.01

Revisiting the role of type 1 conventional dendritic cells (cDC1) in cancer immunosurveillance and in responses to immunotherapies against cancer

R. Mattiuz[1], *J. Cancel*[1], *L. Pouyet*[2], *F. Romagné*[2], *N. Bendriss-Vermare*[3], *C. Caux*[3], *J. Valladeau-Guilemond*[3], *M. Dalod*[1], *K. Crozat*[1];
[1]Centre d'Immunologie de Marseille Luminy, Marseille, France, [2]MI-mAbs, Marseille, France, [3]Centre de recherche en cancérologie - Lyon, Lyon, France.

It is essential to develop new clinical strategies to reactivate the natural anti-tumoral CD8+ T cell responses, or to induce de novo protective immunity in cancer patients. Here, we aim at analyzing to what extent type 1 conventional dendritic cells (cDC1) functions can be manipulated to improve immunotherapies currently applied to cancer. cDC1 excel in tumor antigen cross-presentation, and are strong inducers of CD8+ T cell responses in many experimental settings. In human, tumor infiltrating cDC1 are associated with a good prognosis in many solid cancers. Therefore, the protective functions of cDC1 need to be described both in context of immunotherapies against cancers and during natural tumor immuno-surveillance to tumor. We have generated unique mouse models, which allow either a constitutive or a conditional depletion of all cDC1 through the body. Using these mice, we found quite unexpectedly, that cDC1 are dispensable for tumor control at the time of immunotherapy administration (ACT or checkpoint blockers) in thymoma, melanoma and colon cancer models. However, cDC1 are critical to tumor immunosurveillance in a model of breast cancer that is naturally controlled when orthotopically engrafted in mice. In this last setting, cDC1 protective functions depend on anti-tumor CD8+ T cells and their infiltration into the tumor. Although our work puts in perspective recent findings about the involvement of cDC1 in immunotherapies against cancer, it will nonetheless help in defining new ways to mobilize cDC1 functions to improve immunotherapies currently applied in clinics to patients.

WS.B1.06.02

Generation of Memory Stem T cells specific for tumor antigens and resistant to inhibitory signals by genome editing

B. C. Cianciotti[1], *A. Potenza*[1], *Z. Magnani*[1], *V. Vavassori*[2], *P. Genovese*[2], *L. Naldini*[2], *E. Ruggiero*[1], *C. Bonini*[1];
[1]Division of Immunology, Transplantation, and Infectious Diseases, Experimental Hematology Unit, Ospedale San Raffaele, Milan, Italy, [2]San Raffaele Telethon Institute for Gene Therapy (TIGET), Ospedale San Raffaele, Milan, Italy.

Introduction: Exhausted T cells infiltrating solid tumors express multiple inhibitory receptors (IRs) that are exploited by cancer cells for protection from immune attack. We recently showed that a large fraction of $CD8^+$ cells infiltrating the bone marrow of patients with acute myeloid leukemia relapsing after allogeneic transplantation co-express IRs. Here we aim to simultaneously redirect T cell specificity and permanently disrupt IRs by CRISPR/Cas9 system in long-living memory stem T cells (T_{SCM}) for adoptive cell therapy.
Methods: Primary T cells activated with anti-CD3/CD28-conjugated beads and cultured with IL7+IL15 were electroporated with Cas9/gRNA ribonucleoproteins (RNPs) targeting Tim3, LAG3, 2B4 and the TCR alpha chain constant region (TRAC) genes. The frequency of NHEJ was assessed with FACS analysis, surveyor assay and ddPCR. A lentiviral vector (LV) encoding for an NY-ESO1-specific TCR (LV-NYESO1-TCR) was used for TCR gene editing.
Results: By single genome editing we obtained up to 60-70% genetic disruption at the Tim3 locus, 80-90% at the LAG3 and 63% at 2B4 locus. TRAC gene disruption resulted 98% $CD3^{neg}$ cells, that were then efficiently (70-85%) transduced with LV-NYESO1-TCR. We then combined one IR disruption with TCR gene editing in a single protocol. We obtained an average of 47% and 69% of $Tim3^{neg}$ and $LAG3^{neg}$ NY-ESO1-TCR redirected T cells respectively. More than 90% of edited cells showed a T_{SCM} functional phenotype and proved effective and specific in killing $NY\text{-}ESO1^+$ tumor targets.
Discussion: By exploiting the plasticity and multiplexity of CRISPR/Cas9 we generated innovative tumor-specific cellular products resistant to inhibitory signals.

WS.B1.06.03
A CD3xCD19 bispecific DART®molecule induces T-cell mediated killing of CLL cells *in vitro*

A. W. J. Martens[1], *S. R. Janssen*[1], *L. Izhak*[2], *A. P. Kater*[1], *G. J. van der Windt*[1];
[1]*Academic Medical Center, Amsterdam, Netherlands,* [2]*The Janssen Pharmaceutical Companies of Johnson & Johnson, Spring House, Pennsylvania, United States.*

Chronic lymphocytic leukemia (CLL) is an incurable B-cell malignancy, associated with severe T-cell dysfunction. Recently, CD3xCD19 bispecific antibodies have been successfully applied in lymphoblastic leukemia, but efficacy in CLL has not been thoroughly assessed. We investigated whether CLL cells can be targeted by CD3xCD19 bispecific DART® (also known as MGD011) therapy and if this overcomes T-cell dysfunction in CLL.
We found that MGD011 redirects healthy donor T-cells to kill CD19$^+$ lymphoma cell lines, and primary CLL cells. Clinical responses seen in current therapies are highly dependent on specific prognostic factors (unmutated IgVH, high-risk cytogenetics, and chemoresistance). However, using MGD011, killing of CLL cells could be achieved at comparable levels among these different prognostic subgroups.
Next, we assessed the responses of CLL-derived T-cells upon therapy with MGD011. When autologous CLL cells were used as targets, addition of MGD011 resulted in robust activation of both CD4$^+$ and CD8$^+$ cells, measured by CD25 upregulation, proliferation, TNFα and IFNγ production and degranulation. CLL-derived T-cells (CD4 vs CD8 vs combined) were able to kill CD19$^+$ cell lines (average specific lysis at a 1:1 E:T ratio of 85%). Killing of autologous CLL by single CD4 or CD8 cells was remarkably lower but 1:1 combination of CD4 and CD8 resulted in enhanced killing capacity (21%, 25% and 45% for single CD4, single CD8 and combined respectively).
Together, this indicates that even high-risk CLL cells can be killed using MGD011. Nevertheless, we are currently investigating means to further improve the T-cell mediated lysis of CLL cells.

WS.B1.06.04
MDM2-targeting as a new Natural Killer cell-based immunotherapy of neuroblastoma

I. Veneziani[1], *E. Ferretti*[2], *D. Fruci*[1], *L. Moretta*[3], *V. Pistoia*[3], *F. Locatelli*[1], ***L. Cifaldi***[1];
[1]*Department of Pediatric Hematology and Oncology, Bambino Gesù Children's Hospital, Rome, Italy,* [2]*Laboratory of Oncology, Giannina Gaslini Institute, Genoa, Italy,* [3]*Immunology Research Area, Bambino Gesù Children's Hospital, Rome, Italy.*

Neuroblastoma (NB) is the most common extracranial solid tumor occurring in childhood. Amplification of the MYCN oncogene is associated with poor prognosis. Down-regulation of ligands on tumor cells recognized by Natural Killer (NK) cell-activating receptors, involved in tumor cell recognition and lysis, may contribute to tumor progression and relapse. We demonstrated that MYCN expression is inversely correlated with ligands recognized by NKG2D and DNAM1-activating receptors. ULBP activating ligands are induced by p53 and c-MYC transcription factors. Of note, p53 is mutated only in 2% of NB. On the other hand, MDM2 inhibits p53 function. Both p53 and MDM2 are direct transcriptional targets of MYCN and are co-expressed at high levels in MYCN-amplified NB cells. Indeed, p53 is functionally suppressed by MDM2 in MYCN-amplified NB cells. In this study, we found that MDM2-targeting mediated by Nutlin-3a, a well-established MDM2 antagonist, enhanced *in vitro* the expression of activating ligands in NB cells, thus rendering them more susceptible to NK cell-mediated killing, and *in vivo* in NSG-mice-xenograft model. Moreover, ChIP assay revealed that p53, upon Nutlin-3a treatment, is a direct transcription factor of PVR. Taken together, these results provide the first demonstration that MYCN acts as an immunosuppressive oncogene in NB cells. The restoration of p53 function, mediated by MDM2-targeting, represents a promising molecular approach to sensitize NB cells to NK cell-mediated killing to be exploited to design a new NK cell-based immunotherapy to treat high-risk NB patients. *Grant: Italian Ministry of Health (Rome, Italy) grant GR-2011-02352151 to Loredana Cifaldi.*

WS.B1.06.05
Mutated *NPM1* as target for immunotherapy of acute myeloid leukemia

D. I. van der Lee*, R. M. Reijmers, M. W. Honders, R. S. Hagedoorn, R. M. de Jong, M. G. Kester, D. M. van der Steen, A. H. de Ru, H. M. Bijen, C. Kweekel, I. Jedema, H. Veelken, M. H. Heemskerk, P. A. van Veelen, F. J. Falkenburg, M. Griffioen;*
Leiden University Medical Center, Leiden, Netherlands.

The most frequent subtype of acute myeloid leukemia (AML) is defined by mutations in the nucleophosmin (*NPM1*) gene. Mutated *NPM1* is an attractive target for immunotherapy, since it is an essential driver gene and 4 base pair frameshift insertions occur in the same region in 30% of AML, resulting in a novel C-terminal alternative reading frame of 11 amino acids. By searching in the HLA class I ligandome of primary AML, we identified multiple peptides derived from mutated *NPM1*. For one of these peptides, i.e. HLA-A*02:01-presented CLAVEEVSL, we searched for specific T-cells in healthy individuals using peptide-MHC tetramers. Tetramer-positive CD8 T-cell clones were isolated and analyzed for reactivity against primary AML with mutated *NPM1*. From one selected clone with superior anti-tumor reactivity, we isolated the T-cell receptor (TCR) and demonstrated specific recognition and lysis of HLA-A*02:01-positive AML with mutated *NPM1 in vitro* after retroviral transfer to CD8 and CD4 T-cells. Anti-tumor efficacy of TCR-transduced CD8 and CD4 T-cells was confirmed in a mouse model engrafted with a human AML cell line expressing mutated *NPM1*. Interestingly, co-infusion of TCR-transduced CD8 and CD4 T-cells resulted in superior anti-tumor reactivity as compared to TCR-transduced CD8 T-cells alone.
These data show that mutated *NPM1*-derived peptides are presented on AML and that CLAVEEVSL is a neoantigen that can be efficiently targeted on AML with mutated *NPM1* by TCR gene transfer in a co-receptor independent fashion. Immunotherapy targeting mutated *NPM1* may therefore contribute to treatment of AML. This research was supported by the Dutch Cancer Society.

WS.B1.06.06
Enhanced immunogenicity of mitochondrial localised mutated proteins in cancer cells

G. Prota[1], *U. Gileadi*[1], *M. Rei*[1], *A. Lechuga Vieco*[2], *J. Chen*[1], *J. Rehwinkel*[1], *A. Ahmed*[3], *J. Enríquez*[2], *V. Cerundolo*[1];
[1]*MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, Oxford, United Kingdom,* [2]*Centro Nacional de Investigaciones Cardiovasculares Carlos III (F.S.P.), Madrid, Spain,* [3]*Nuffield Department of Obstetrics and Gynaecology, University of Oxford, UK, Oxford, United Kingdom.*

The evidence that priming of tumour specific CD8+ T cells relies on cross-presentation of tumour debris by dendritic cells (DCs) is compelling. However, it remains unclear whether cellular localisation of mutated cancer proteins plays a role in the ability of CD8+ T cells to be primed by cancer cells. To address this question, we have analysed the ability of the full length NY-ESO-1 protein and ovalbumin localised either in mitochondria or cytosol to elicit *in vivo* antigen specific CD8+ T cell responses and compared direct vs cross-presentation. Our results show that cytoplasmic proteins are rapidly degraded and elicit strong direct presentation *in vitro* of processed epitopes. In contrast, mitochondrial localised proteins are significantly longer lived, due to their protection from proteasome dependent degradation, and are capable of eliciting *in vivo* significantly stronger antigen specific CD8+ T cell responses than cytosolic proteins. We showed that such enhanced response of mitochondrial localised proteins is mediated by cross-presentation events dependent on STING activation and by uptake of mitochondria by CD103+ and CD11b+ DC. Finally, we have extended these findings in humans by isolating several CD8+ T cells clones specific to defined epitopes expressed by mitochondrial localised proteins from one endometrial cancer patient with high mutational burden due to loss of function of the proof reading polymerase POLE. In conclusion, these findings demonstrate the greater immunogenicity of mitochondrial localised mutated proteins, highlighting strategies to identify highly immunogenic peptide epitopes for the development of cancer vaccination strategies.

WS.B2.01 Environmental regulation of anti-tumor responses

WS.B2.01.01
High glycolytic tumors evade immune destruction by acidosis-dependent induction of ICER in tumor-associated macrophages

T. Bohn[1], *S. Rapp*[2], *M. Klein*[1], *S. Pektor*[3], *K. Renner*[4], *M. Kreutz*[4], *V. Popp*[5], *K. Gerlach*[5], *B. Weigmann*[5], *C. Lueckel*[6], *M. Huber*[6], *C. Becker*[7], *E. von Stebut-Borschitz*[8], *H. Schild*[1], *E. Schmitt*[1], *T. Bopp*[1];
[1]*Institute for Immunology, Mainz, Germany,* [2]*Institute for Preventive Cardiology, Mainz, Germany,* [3]*Institute for Nuclear Medicine, Mainz, Germany,* [4]*Internal Medicine III, Regensburg, Germany,* [5]*Department of Medicine 1, Erlangen, Germany,* [6]*Institute for Medical Microbiology and Hospital Hygiene, Marburg, Germany,* [7]*Dermatology, Mainz, Germany,* [8]*Dermatology, Köln, Germany.*

Aggressive types of skin cancer have become more common over the last 25 years. Immune checkpoint inhibitors have revolutionized melanoma treatment. However, only less than 40% of patients benefit from this therapy suggesting additional immune evasion mechanisms. To develop new innovative therapeutic strategies, detailed understanding of tumor- and micro milieu-specific mechanisms contributing to inefficient anti-tumor immune responses is essential. Therefore, we focused our work on identifying signaling pathways and molecules involved in melanoma formation and anti-tumor immune responses.
We explored a molecular mechanism of non-classical physicochemical communication deployed by high glycolytic tumors for immune evasion. Melanomas are transcriptionally privileged to produce energy by high-rate glycolysis resulting in tumor acidification. This tumor acidosis induced a cAMP-mediated expression of the transcriptional repressor ICER in tumor-associated macrophages (TAM), leading to functional polarization of TAM towards a non-inflammatory M2 phenotype promoting tumor growth. In contrast to that, B16 melanoma-infiltrating macrophages of *Icer*-deficient mice possess an inflammatory anti-tumor M1 phenotype which results in a spontaneous rejection of high glycolytic tumors by *Icer*-deficient mice, as well as mice with a conditional deficiency of *Icer* in macrophages.
To test the "drugability" of cAMP modulation in melanoma treatment we conducted *in vivo* experiments with therapeutic application of the Adenylate cyclase inhibitor MDL-12. Inhibition of *de novo* cAMP synthesis lead to a rejection of B16 melanomas in C57BL/6J mice.
Taken together, our findings identify an evolutionary conserved mechanism of physicochemical communication between non-lymphoid tissue and the immune system that is exploited by high glycolytic tumors for immune evasion.

WS.B2.01.02

Carcinoma-associated pancreatic stellate cells induce CD4$^+$ and CD8$^+$ T-cell exhaustion

L. Gorchs[1], *C. Fernández Moro*[2], *Q. Meng*[1], *E. Rangelova*[3,4], *H. Kaipe*[1,5];
[1]*Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden,* [2]*Department of Pathology/Cytology, Karolinska University Hospital, Stockholm, Sweden,* [3]*Department of CLINTEC, Karolinska Institutet, Stockholm, Sweden,* [4]*Pancreatic Surgery Unit, Center for Digestive Diseases, Karolinska University Hospital, Stockholm, Sweden,* [5]*Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden.*

Carcinoma-associated pancreatic stellate cells (CAPSCs) are the major type of stromal cells in pancreatic ductal adenocarcinomas and besides their pathological release of extracellular matrix proteins they are also perceived as key contributors to immune evasion. Despite the known relevance of tumor infiltrating lymphocytes in cancers, the interactions between T-cells and CAPSCs remain largely unexplored. Here, we found that CAPSCs isolated from tumors of pancreatic cancer patients expressed higher levels of the PD-1 ligands PD-L1 and PD-L2 compared to primary skin fibroblasts from healthy donors. CAPSC strongly inhibited T-cell proliferation via both contact-dependent and independent fashions. Blocking the activity of prostaglandin E_2 (PGE_2) partially restored the proliferative capacity of both CD4$^+$ and CD8$^+$ T-cells. After stimulation, the proportion of proliferating T-cells expressing HLA-DR and the proportion of memory T-cells was decreased when CAPSCs were present compared to T-cells proliferating in the absence of CAPSC. Interestingly, proliferating T-cells had a greater expression of the immune checkpoint markers TIM-3, PD-1, CTLA-4 and LAG-3 in the presence of CAPSCs. Functional assays showed that T-cells expressing immune checkpoints produced less IFN-γ, TNF-α, and CD107a after restimulation when CAPSCs had been present. Thus, this indicates that CAPSCs induce expression of immune checkpoints on CD4$^+$ and CD8$^+$ T-cells, which contribute to a diminished immune function. This study was funded by the Swedish Cancer Society (Dnr 2017/749) and the Cancer Society in Stockholm (Dnr 171083).

WS.B2.01.03

The tumour microenvironment supports the self-renewal of tumour-promoting macrophages in colon adenoma

I. Soncin*, J. Sheng, Q. Chen, K. Karjalainen, C. Ruedl;*
Nanyang Technological University, Singapore, Singapore.

Circulating CCR2$^+$ monocytes are crucial in maintaining the adult tissue-resident F4/80^{hi}MHCII^{hi} macrophage pool in the intestinal lamina propria.
Herein, we delineated murine colonic tissue-resident F4/80^{hi} macrophages and identified a CCR2-independent F4/80^{hi}MHCII^{lo} macrophage subset as the only F4/80^{hi} subset present in the embryonic lamina propria that was gradually lost after birth and almost entirely replaced by CCR2-dependent F4/80^{hi}MHCII^{hi} macrophages in the adulthood. However, in colon adenomas F4/80^{hi}MHCII^{lo} macrophages were not only preserved but became the dominant tumour-associated macrophages within large tumours. Furthermore, by utilizing Kit$^{MerCreMer/R26}$ fate-mapping mouse model we demonstrated that in the tumour microenvironment both macrophage fractions were able to maintain their numbers mostly independent from bone marrow contribution. Indeed, they upregulated the expression of numerous genes related with cell proliferation, which may represent the driving source of *in situ* maintenance. Analyses of colon adenomas indicated that CSF1 could be a key facilitator of macrophage self-renewal.
Thus, as the intestinal environment switches from healthy to tumoural there is a corresponding shift in the renewal of tissue-resident macrophages, from bone-marrow dependency to self-maintenance through *in situ* proliferation, conferred by the local niche.
This work was supported by MOE2014-T2-1-011 and MOE2016-T2-1-012 Ministry of Education Tier2 grants to C.R.

WS.B2.01.04

Fatty acid metabolism complements glycolysis in the selective regulatory T cell expansion during tumor growth

I. Pacella[1], *C. Procaccini*[2], *C. Focaccetti*[1], *S. Miacci*[1], *E. Timperi*[1], *D. Faicchia*[3], *M. Severa*[4], *F. Rizzo*[4], *E. M. Coccia*[4], *F. Bonacina*[5], *N. Mitro*[5], *G. D. Norata*[5,6], *G. Rossetti*[7], *V. Ranzani*[7], *M. Pagani*[7,5], *E. Giorda*[8], *Y. Wei*[9], *G. Matarese*[2,3], *V. Barnaba*[1,10], *S. Piconese*[1,10];
[1]*Sapienza Università di Roma, Rome, Italy,* [2]*Consiglio Nazionale delle Ricerche (IEOS-CNR), Naples, Italy,* [3]*Università di Napoli "Federico II", Naples, Italy,* [4]*Istituto Superiore di Sanità, Rome, Italy,* [5]*Università degli Studi di Milano, Milan, Italy,* [6]*School of Pharmacy and Biomedical Sciences, Curtin University, Perth, Australia,* [7]*Istituto Nazionale Genetica Molecolare INGM 'Romeo ed Enrica Invernizzi', Milan, Italy,* [8]*Ospedale Pediatrico Bambino Gesù IRCCS, Rome, Italy,* [9]*Institut Pasteur Paris, Paris, France,* [10]*Istituto Pasteur Italia-Fondazione Cenci Bolognetti, Rome, Italy.*

Recent studies have established that metabolic restrains, such as glucose restriction, impair the activities of effector T cells in the tumor microenvironment. In the same context, a huge expansion of activated regulatory T cells (Tregs) in tumor tissues has been described in mice and humans, contributing to the suppression of protective anti-tumor immunity. Here we showed that Tregs advantage in the tumor milieu relies on supplemental energetic routes involving lipid metabolism.
In murine models, tumor-infiltrating Tregs displayed intracellular lipid accumulation, which was attributable to an increased rate of fatty acid (FA) synthesis. Since relative advantage in glucose-uptake may fuel FA synthesis in intratumoral Tregs, we demonstrated that both glycolytic and oxidative metabolism concurred to Tregs expansion. We corroborated our data in human tumors showing that Tregs displayed a gene signature towards glycolysis and lipid synthesis. Our data support a model in which signals from the tumor microenvironment induce a circuitry of glycolysis, FA synthesis and oxidation that confers to Tregs a preferential proliferative advantage compared to the counterpart effector T cells that rely primarily on the glycolytic pathway for their metabolic demands. The awareness of the metabolic dynamics of Treg in tumor could provide new means for cancer immunotherapy.

WS.B2.01.05

IL-18 is a key driver of immunosuppression and a therapeutic target in multiple myeloma

S. Kassem[1], *K. Nakamura*[2], *H. Avet-Loiseau*[3], *M. J. Smyth*[2], *L. Martinet*[3];
[1]*Cancer Research Center of Toulouse, TOULOUSE, France,* [2]*QIMR Berghofer, Brisbane, Australia,* [3]*Cancer Research Center of Toulouse, Toulouse, France.*

Tumor-promoting inflammation and avoiding immune destruction are hallmarks of cancer. Here, we demonstrate that the pro-inflammatory cytokine IL-18 is critically involved in these hallmarks in multiple myeloma (MM). Mice deficient for IL-18 were remarkably protected from Vk*MYC MM progression in a CD8$^+$ T cell-dependent manner. The MM-niche derived IL-18 drove generation of myeloid-derived suppressor cells (MDSCs), leading to accelerated disease progression. A global transcriptome analysis of the immune microenvironment in 73 MM patients strongly supported the negative impact of IL-18-driven MDSCs on T cell responses. Strikingly, high levels of bone marrow plasma IL-18 were associated with poor overall survival in MM patients. Furthermore, our preclinical studies suggested that IL-18 could be a potential therapeutic target in MM.
Although novel anti-myeloma agents have been developed, MM is still an incurable disease. The growth of MM cells highly depends on the bone marrow (BM), where cellular components and soluble factors cooperatively orchestrate the pro-survival and immunosuppressive microenvironment. However, the molecular and cellular mechanisms of MM-associated inflammation and immunosuppression remain poorly understood. Using Vk*MYC preclinical models, a comprehensive analysis of the transcriptional landscape of the immune microenvironment in MM patients, and BM
cytokine analyses, we demonstrate that dysregulated production of IL-18 is a key driving force for immunosuppression in the MM microenvironment and a potential therapeutic target. Our results reveal the critical role of IL-18 in the MM immunopathology, which provides insight into therapeutic strategies against MM (Nakamura et al. Cancer Cell, 2018).

WS.B2.01.06

Chronic lymphocytic leukemia impairs metabolic fitness in CD8 T cells

A. van Bruggen[1], *A. Martens*[1], *J. A. Fraietta*[2], *M. Levin*[3], *P. J. Siska*[4], *S. Endstra*[1], *J. C. Rathmell*[4], *J. J. Melenhorst*[2], *A. P. Kater*[1], *G. J. van der Windt*[1];
[1]*Academic Medical Center, Amsterdam, Netherlands,* [2]*University of Pennsylvania, Philadelphia, United States,* [3]*Albert Schweitzer Hospital, Dordrecht, Netherlands,* [4]*Vanderbilt University Medical Center, Nashville, United States.*

In chronic lymphocytic leukemia (CLL) T cell exhaustion is acquired and impedes development of effective immunotherapeutic strategies by yet unknown mechanisms. Since cellular metabolism is tightly linked to T cell function, we hypothesized that interaction of CLL cells with T cells leads to an altered metabolic programming resulting in impaired T cell function. Here we used CLL-derived and age-matched healthy donor (HD)-derived PBMCs, and CAR-T cell infusion products derived from CLL patients to study CD8 T cell metabolism. CLL-derived CD8 T cells showed increased mitochondrial membrane potential and basal respiration, while spare respiratory capacity was reduced, indicating a lower ability to deal with enhanced energy demand. In addition, CD8 T cells in CLL had increased mitochondrial ROS, which coincided with decreased levels of PGC1a and the anti-oxidant super oxide dismutase 2. After stimulation in vitro, CLL CD8 T cells became less activated, showed decreased glucose uptake and glycolysis, and impaired mitochondrial biogenesis. In line with this, antigen experienced CD8 T cells in CLL showed no increase in mitochondrial mass compared to naïve CD8 T cells, as opposed to healthy donors. CD8 CAR-T cells from complete responders had higher mitochondrial mass compared to non-responders which correlated positively with the expansion of the CAR-T cell culture in vitro, and CAR-T cell persistence in vivo. These data indicate that metabolic fitness of CD8 T cells is impaired in CLL. Therefore, boosting T cell metabolism in CLL by enhancing mitochondrial biogenesis might improve CAR-T cell therapy and other emerging immunotherapies.

WS.B2.02 Tumor immune surveillance and evasion

WS.B2.02.01
Tumor immune evasion arises through loss of TNF sensitivity

***J. Oliaro**, C. Kearney, S. Vervoort, R. Johnstone;*
Peter MacCallum Cancer Centre, Melbourne, Australia.

Immunotherapy has revolutionized outcomes for cancer patients, but the mechanisms of resistance remain poorly defined. Here, we used a series of whole genome CRISPR-based screens performed *in vitro* and *in vivo* to identify mechanisms of tumor immune evasion from cytotoxic lymphocytes ($CD8^+$ T cells and natural killer (NK) cells). Deletion of key genes within the TNF signaling, IFN-γ signaling, and antigen presentation pathways provided protection of tumor cells from $CD8^+$ T cell-mediated killing, and blunted anti-tumor immune responses *in vivo*. Deletion of a number of genes in the TNF pathway also emerged as the key mechanism of immune evasion from primary NK cells. Remarkably, we found that tumors delete the same genes when exposed to perforin deficient $CD8^+$ T cells, demonstrating that the dominant immune evasion strategy utilized by tumor cells is acquired resistance to T cell-derived cytokine mediated anti-tumor effects. Indeed, we demonstrate that TNF-mediated bystander killing is a potent T cell effector mechanism capable of killing antigen-negative tumor cells. In addition to highlighting the importance of TNF in $CD8^+$ T and NK cell mediated killing of tumor cells, our study also provides a comprehensive picture of the roles of the TNF, IFN and antigen presentation pathways in immune-mediated tumor surveillance.

WS.B2.02.02
IL-33/ST2 signaling promotes the survival and proliferation of AML1/ETO leukemic stem cells

***P. Näf**[1], R. Radpour[1], C. Riether[1,2], A. F. Ochsenbein[1,2];*
[1]Department for BioMedical Research, Bern, Switzerland, [2]Department of Medical Oncology, Bern, Switzerland.

Interleukin (IL)-33 is an alarmin released upon cell necrosis and binds to the heterodimeric receptor ST2/IL1RaP, which is expressed on a subset of immune and epithelial cells. BCR/ABL transformation in chronic myeloid leukemia (CML) stem cells was shown to induce ST2 expression and propel malignant cell growth. However, the role of IL-33/ST2 signaling in acute myeloid leukemia (AML) is currently unknown. Gene expression analysis of human bulk and $CD34^+CD38^+$ AML samples harboring variety of translocations revealed that ST2 was particularly upregulated on AML1/ETO transformed AML cells, but not expressed on other AML subtypes and healthy hematopoietic stem cells (HSCs). We next generated an *in vivo* AML model by transfecting murine hematopoietic stem cells with an AML1/ETO lentiviral construct. AML/ETO transformed leukemic stem cells (LSCs) highly expressed ST2 and showed increased colony formation in recombinant IL-33 (rIL-33) conditioned methylcellulose-based culture assays when compared to controls. Interestingly, amongst all screened human AML1/ETO LSCs a heterogeneity in ST2 expression was observed. Molecular profiling of the LSCs revealed a negative correlation of ST2 gene expression and key genes of the Notch pathway. Blocking the Notch pathway in the $ST2^{low}$AML1/ETO LSCs using siRNA revealed that ST2 protein expression could be restored. Furthermore, siRNA induced gain of ST2 expression also restored *in vitro* colony formation capacity in the presence of rIL-33. Taken together, these data provide evidence that IL-33/ST2 signaling plays a disease promoting role in AML1/ETO transformed AML LSCs. Further investigations will focus on detailed signaling mechanisms and propose new therapeutic strategies to treat AML.

WS.B2.02.03
Characterizationof CC-chemokine Receptor 6 (CCR6) and CC-chemokine Ligand 20 (CCL20) mediated immunosurveillance in the initiation and progression of malignant melanoma

***D. Martin-Garcia**, A. H. Enk, A. S. Lonsdorf;*
Department of Dermatology, Heidelberg, Germany.

Chemokine ligand 20 (CCL20) expressed in the epidermis is a potent impetus for the recruitment of subsets of DCs, B-cells and memory T-cells expressing chemokine receptor 6 (CCR6), its exclusive receptor. CCL20 and a corresponding CCR6-expressing immune cell infiltrate have been detected in several malignancies, including melanoma. Yet, the functional contribution of the CCR6/CCL20 axis for the immune control of melanoma remains controversial. The characterization of CCR6-guided immune cell subsets and their functional contribution for the immune control of melanoma comprises the focus of this project. We evaluated the homeostatic and inducible secretion of CCL20 by different murine and human melanoma cutaneous cell lines by ELISA. Both murine (B16, Ret) and human (A375, C32) melanoma cell lines are capable of secreting CCL20 upon stimulation with pro-inflammatory cytokines in vitro. In order to determine the functional relevance of CCR6 on local tumor growth, B16 melanoma cells retrovirally transduced with a vector that constantly overexpresses CCL20 (B16-CCL20) were injected subcutaneously in C57BL/6 wt mice and congenic CCR6-knockout (CCR6ko) mice. While animals in both groups developed local tumors, we observed a significantly reduced tumor growth in CCR6ko mice. By contrast, wt and CCR6ko control groups did not display differences in tumor growth rate. Our results suggest that CCL20 interactions in the microenvironment of cutaneous melanoma may be an essential factor for local tumor growth. Preliminary experiments have pointed out a possible autocrine pathway that would affect only B16 tumor growth in CCR6ko mice, although the precise mechanisms are still being investigated.

WS.B2.02.04
Unraveling the mechanism behind aberrant CD37 expression in human B cell lymphoma

***S. Elfrink**[1], C. de Winde[1], M. van den Brand[2], E. Jansen[1], F. Doubrava-Simmer[2], S. van Deventer[1], C. Hess[3], W. Stevens[3], D. van Spronsen[3], H. van Krieken[2], C. Figdor[1], B. Scheijen[2], A. van Spriel[1];*
[1]Department of Tumor Immunology, Radboud Institute of Molecular Life Sciences, Radboudumc, Nijmegen, Netherlands, [2]Department of Pathology, Radboudumc, Nijmegen, Netherlands, [3]Department of Hematology, Radboudumc, Nijmegen, Netherlands.

Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma and remains a clinical problem. We and others have recently shown loss of immune cell-specific tetraspanin CD37 protein in half of the DLBCL tumors, which is directly correlated with clinical outcome. Tetraspanins are membrane proteins that control cell surface organization by forming tetraspanin enriched microdomains. Hereby they are involved in cell migration, proliferation and survival, and play a role in cancer cell migration and cellular interactions. We discovered that CD37 controls signal transduction pathways that regulate the survival of B cells, and *Cd37*-deficient mice spontaneously develop lymphomas. Genomic mutation analysis of the coding sequence of the human *CD37* gene revealed a pathogenic mutation in 3/16 DLBCL tumors, resulting in aberrant expression of the protein. Other potential mechanisms of loss of CD37 expression may include alterations in the *CD37* promoter region or increased DNA methylation. Mutation analysis of the promoter region of CD37-negative tumors (n=12) did not reveal any pathogenic mutations. MspJI digestion on genomic DNA indicates increased methylation of the *CD37* promoter region in CD37-negative B cell lines compared to CD37-positive B cell lines. Preliminary results from bisulfite sequencing of the promoter region and the first intron of *CD37* show differential methylation in CD37-expressing cells versus CD37-negative cells. Taken together, the B cell tetraspanin CD37 is a novel tumor suppressor that protects against malignant transformation of B cells. This work provides important insight into the molecular mechanisms underlying CD37-deficiency in human B cell lymphoma.

WS.B2.02.05
A generic, cost-efficient high performance capillary liquid chromatography-high resolution mass spectrometry method for quality control of peptide pools

***G. Bosc-Bierne**[1], V. Armuzza[2], M. G. Weller[1], O. J. Kreuzer[2];*
[1]Federal Institute for Materials Research and Testing (BAM), Berlin, Germany, [2]peptides&elephants GmbH, Berlin, Germany.

Synthetic peptide pools are used in antigen-specific T-cell assays, which are an important part in vaccine and immunotherapeutic clinical trials. As the analytical characterization is challenging due to the similarity of the single peptides or is expensive due to isotope labeled standards, usually only a pre-characterization of the single peptides is performed. However, a regular quality control of the peptide mix would be highly desirable. Therefore, a cost-efficient high performance liquid chromatography-high resolution mass spectrometry (HPLC-HRMS) method for quality control of a model peptide pool is developed.
Peptides were synthesized using peptides&elephants proprietary libraries of individual peptides (LIPS) technology and purified by reversed-phase chromatography to >90% each. The lyophilized single peptides were combined to a model peptide pool and analyzed by reversed-phase high-performance capillary liquid chromatography coupled to an orbitrap mass spectrometer. Separation was performed on a capillary reversed phase column (2 µm, ID x L 300 µm x 150 mm) with a linear gradient of acetonitrile + 0,05 % trifluoroacetic acid. Absolute quantification was accomplished based on ultraviolet spectroscopy. Identification was obtained by high resolution mass spectrometry.
After optimizing the injection mode, the gradient elution, the temperature, the additives and sample preparation a model peptide pool was separated. The extracted ion chromatogram (XIC) was studied to confirm the exact masses as well as the total ion chromatogram (TIC) to identify possible degradation products.
By combination of capillary HPLC and HRMS a new cost-efficient quality control method could be developed for the separation and quantification of complex synthetic peptide pools.

WS.B2.02.06
G-MDSC derived exosomes promote the progression of colon cancer

S. WANG[1,2], *Y. WANG*[1], *J. MA*[3], *H. XU*[3];
[1]*THE AFFILIATED PEOPLE'S HOSPITAL, JIANGSU UNIVERSITY, ZHENJIANG, China,* [2]*Department of Immunology, School of Medicine, Jiangsu University, Zhenjiang, China,* [3]*Department of Immunology, School of Medicine, Jiangsu University, ZHENJIANG, China.*

Introduction: Myeloid-derived suppressor cells (MDSC) are present in most cancer patients where these cells inhibit anti-tumor immunity and enhance cancer stem cells (CSC) which are important cellular component in the cancer microenvironment. MDSC derived exosomes have been proposed to act as intercellular communicators, but the role of MDSC derived exosomes in colon cancer is unclear.
Materials and Methods: Granulocytic MDSCs were isolated by microbeads, and G-MDSC derived exosomes (GM-Exo) were purified from the cell supernatant. Phenotypic analysis of immune cells was performed by flow cytometry. Cancer cell stemness was detected by sphere formation and biomarkers. Tumor model were monitored and assessed by histopathology.
Results: GM-Exo promoted colon carcinoma CT-26 cells proliferation and migration in vitro. GM-Exo exacerbated tumor incidence and developed rapidly progressing lethal tumors mice, and enhanced the migration ability of CT-26 cells through liver metastasis model in vivo. Exosomal S100A9 enhanced tumor sphere formation and the levels of a panel of established CSC markers, which was associated with Nox/ROS/NF-κB pathway. After treatment of GM-Exo, the percentage of CD133+ or CD44+ CSC cells was increased in tumor tissue. Silencing of Rab27a halted G-MDSC exosomes release, and attenuated the ability to promote the development of transplanted tumor.
Conclusions: The results suggested that S100A9 cargo packaged into GM-Exo promoted the metastatic and tumorigenic potential of colon cancer through enhancing cancer cell stemness.

WS.B2.03 Innate anti-tumor immunity

WS.B2.03.01
The macrophage tetraspan MS4A4A interacts with Dectin-1 and is required for macrophage-NK cell cross-talk and resistance to metastasis

I. Mattiola[1,2], *F. Tomay*[2], *M. De Pizzol*[2], *B. Savino*[2], *T. Gulic*[3], *R. Silva-Gomes*[4], *R. Carriero*[2], *D. Morone*[2], *I. N. Shalova*[5], *Y. Lee*[5], *S. K. Biswas*[5], *G. Mantovani*[6], *M. Sironi*[2], *B. Bottazzi*[2], *A. Mantovani*[2,7,8], *M. Locati*[2,3];
[1]*Department of Microbiology and Infection Immunology, Charité - University Medical Centre Berlin, Berlin, Germany,* [2]*Humanitas Clinical and Research Center, Pieve Emanuele, Italy,* [3]*Department of Medical Biotechnologies and Translational Medicine, University of Milan, Milan, Italy,* [4]*Graduate Program in Areas of Basic and Applied Biology (GABBA), Instituto de Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, Porto, Portugal,* [5]*Singapore Immunology Network (SIgN), Agency for Science, Technology & Research (A-STAR), Singapore, Singapore,* [6]*Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Endocrinology Unit, Department of Clinical Sciences and Community Health, University of Milan, Milano, Italy,* [7]*Humanitas University, Pieve Emanuele, Italy,* [8]*The William Harvey Research Institute, Queen Mary University of London, London, United Kingdom.*

Introduction: MS4A4A is a tetraspan-like molecule that is induced during monocyte-to-macrophage differentiation and is upregulated by M2 or M2-like signals, including IL-4, dexamethasone and tumor cell supernatants. We observed that MS4A4A is expressed by human tissue macrophages and tumor-associated macrophages in human cancers and is induced in monocytes from patients treated with methylprednisolone. However, MS4A4A function is totally unknown. **Materials:** Associating molecules of MS4A4A were identified by a split-ubiquitin assay and then confirmed by FLIM-FRET, co-immunoprecipitation and immunofluorescence. Conditional knock-out mice for MS4A4A ($Ms4a4a^{fl/fl}$ x LysM-Cre) were generated and MS4A4A contribution to macrophage functions was assessed both *in vitro* and in *in vivo* models of cancer. **Results:** MS4A4A associates with the β-glucan receptor Dectin-1 and the heterocomplex translocates to lipid rafts. MS4A4A is essential for the full activation of the Syk pathway downstream Dectin-1. The impaired Syk phosphorylation correlates with the reduced production of cytokines and reactive oxygen species by MS4A4A-lacking macrophages. MS4A4A deficiency in macrophages has no influence on primary tumor growth, but impacts on Dectin-1-driven NK-mediated resistance to metastasis. Indeed, the absence of MS4A4A on macrophages impairs NK cell cytotoxic potential leading to the uncontrolled spread of Dectin-1-dependent metastasis. **Conclusion:** We showed that MS4A4A interacts with Dectin-1 and is essential for innate responses driven by Dectin-1, including NK cell-mediated resistance to metastasis. Therefore, we demonstrated for the first time that Dectin-1 is an associating molecule of MS4A4A and that MS4A4A plays a key role in the regulation of macrophage activation.

WS.B2.03.02
Heparanase is required for dendritic cell-natural killer cell crosstalk and subsequent natural killer cell activation

A. Mayfosh, *N. Baschuk;*
Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, Melbourne, Australia.

Heparanase is a beta-D-endoglucuronidase that cleaves heparan sulphate, a major component of the extracellular matrix and basement membrane. Heparanase expression and function have been documented in some leukocyte populations, but have not been described in natural killer (NK) cells. Here we investigate the importance of heparanase in NK cell activation and function, and the impact on tumour clearance. We first observed that heparanase-knock out (Hpse-/-) mice implanted with EO771.LMB tumour cells presented more metastatic lesions in the lungs compared to WT animals, suggesting an impairment in tumour clearance. Following challenge *in vivo* with the viral RNA mimetic Poly(I:C), NK cells isolated from Hpse-/- mice displayed reduced expression of the NK cell activation markers CD69, NKG2D, CD11b and CD27, reduced interferon □ production and impaired cytotoxicity against EO771.LMB cells *in vitro*. Interestingly, direct cytokine stimulation of Hpse-/- NK cells *in vitro* was sufficient to induce NK cell activation equal to that of WT NK cells. Given that Poly(I:C) signals through DCs to stimulate NK cells *in vivo*, we assessed the ability of Hpse-/- DCs to activate WT NK cells *in vitro*. Indeed, Poly(I:C)- activated Hpse-/- DCs were unable to induce NK cell activation equal to that of WT DCs, suggesting heparanase allows for efficient DC-NK cell crosstalk and subsequent NK cell activation. This finding raises the question of how heparanase inhibitors, currently used as anti-cancer therapies, may affect immune surveillance, and uncovers a new approach to increasing immune cell-crosstalk in immunotherapies.

WS.B2.03.03
CD137 (4-1BB) costimulation counteracts TGF-β1 inhibition of antibody-dependent NK cell responses against HER2+ transformed cells

M. Cabo[1], *R. Lozano-Rodríguez*[1], *M. Ataya*[1], *M. Costa-Garcia*[2], *S. Santana*[1], *M. C. Ochoa*[3], *P. Berraondo*[3], *I. Melero*[3,4], *M. López-Botet*[1,2], *A. Muntasell*[1];
[1]*Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain,* [2]*University Pompeu Fabra (UPF), Barcelona, Spain,* [3]*Centro de Investigación Médica Aplicada (CIMA), Pamplona, Spain,* [4]*Clínica Universitaria de Navarra, Pamplona, Spain.*

NK cell recognition of antibody-coated tumor targets through CD16A triggers antibody-dependent cellular cytotoxicity and the release of IFN-γ and TNF-α. Several observations support the contribution of NK cells to the efficacy of anti-HER2 therapeutic antibodies in breast cancer (BC), and NK cell dysfunction owing to immunosuppressive factors (i.e. TGF-β1) has been related to BC progression in metastatic patients. In this context, strengthening NK cell responses by targeting activating co-receptors is envisaged as a relevant option for enhancing the therapeutic benefit of anti-HER2 mAbs. Among stimulatory co-receptors, CD137 was the TNFRSF member showing the highest induction in microarray analysis of CD16-activated human NK cells, as well as along trastuzumab-dependent NK cell activation in a HER2+ BC *in vitro* model. In this context, CD137 upregulation showed earlier kinetics, larger magnitude and sustained persistence as compared to OX40. Trastuzumab-induced CD137 expression was enhanced by TNF-α signaling through TNFRI/TNFRII, as indicated by the effect of specific blocking mAbs. Agreeing with their proficient TNF-α secretion, CD137 upregulation was greater in NK cells from individuals harboring adaptive NKG2C+ NK cell expansions as compared to those lacking this NK cell subset. Remarkably, CD137 co-stimulation in CD16-activated NK cells by the agonist mAb Urelumab reversed TGF-β1-mediated inhibition in a dose-dependent manner, restoring CD25 (IL2Rα) expression, IL2-dependent proliferation and CD16+ NK cell numbers. Overall, our data reveals CD137 as a costimulatory receptor capable of overcoming TGF-β1 immunosuppression, and provide the rationale for combinatorial strategies including CD137 agonists for broadening anti-HER2 mAb therapeutic efficacy.
Financial_sources: WCR(15-1146); AECC(GCB15152947MELE); PIE ISCIII(PIE 2015/00008).

WS.B2.03.04
B7H6, a ligand for NKp30, is regulated by BRD4 in acute myeloid leukemia cells

A. Baragaño Raneros, ***P. Díaz Bulnes****, R. M. Rodriguez, B. Suárez Álvarez, C. López Larrea;*
Traslational Immunology Laboratory, Instituto de Investigación Sanitaria del Principado de Asturias, Oviedo, Spain.

Acute myeloid leukemia (AML) is a disease with a great morphological and genetic heterogeneity making it difficult to treat. Inhibition of bromodomain and extraterminal proteins (BETs) has been proposed as new therapeutic strategy in AML, but its effect on the innate immune response is unknown. NKp30 is a key activating receptor of NK cells that promotes the recognition and lysis of AML blasts through interaction with its B7-H6 ligand. Here, we examined the effect of BETs inhibitors in the B7-H6 expression and its consequences in the immune recognition. Our results showed that B7-H6 expression is drastically reduced in AML cell lines treated with BETs inhibitors (JQ1, I-BET762) leading to a lesser recognition and lysis mediated by NK cells. Assays of chromatin immunoprecipitation revealed that BRD4, the most extensively studied member of the BETs family, directly binds to the B7-H6 promoter region regulating its expression, whilst JQ1 treatment or BRD4 gene silencing abolish that binding. BRD4 recognizes acetylated lysines in histones and recruits the P-TEFb complex (Cyclin T / CDK9) aimed to phosphorylate the RNAPII, allowing gene transcription. We observed that BRD4 binding to B7-H6 promoter is associated with an enrichment of H3 acetylation and phosphorilation of RNAPII. Inhibition of P-TEFb complex with CDK9 inhibitor (DRB) or the p300 histone acetyltransferase with CBP30 suppressed the B7-H6 expression corroborating those results. Thus, B7-H6 expression in AML cells is regulated by BRD4 protein and treatment with BETs inhibitors could damage the immune recognition mediated by NK cells allowing the tumor progression.

WS.B2.03.05

Identification and functional properties of mature and immature neutrophils in homeostasis, inflammation and cancer

J. B. G. Mackey[1,2], *R. Jackstadt*[2], *T. Jamieson*[2], *A. McFarlane*[2], *X. L. Raffo*[2], *D. Patel*[1], *J. Secklehner*[1,2], *J. Vuononvirta*[1], *R. Snelgrove*[1], *J. Morton*[2,3], *J. Norman*[2,3], *O. J. Sansom*[2,3], *L. M. Carlin*[1,2];

[1]*Imperial College London, London, United Kingdom,* [2]*Cancer Research UK Beatson Institute, Glasgow, United Kingdom,* [3]*University of Glasgow, Glasgow, United Kingdom.*

Neutrophils are a critical component of the innate immune response and are capable of identifying and destroying pathogens as a result of their receptor expression and effector mechanisms. During cancer, severe infection and chronic inflammation increased numbers of neutrophils are produced and released from the bone marrow into the peripheral circulation, many of which are postulated to be developmentally immature. Neutrophil maturation within the bone marrow is essential to their development of distinct granules that are crucial to neutrophil function in host defence. We have identified and developed methods that efficiently distinguish immature from mature neutrophils *in vitro* and *in vivo* that can be utilised for their direct study.
Neutrophils are thought to worsen pathology in cancer and severe infection by both direct mediator production and suppression of other leukocytes. We identified immature Ly6G^{Intermediate} and mature Ly6G^{High} neutrophils by flow cytometry, transgenic reporters and adoptive transfer experiments. We studied localisation and function of these immature populations in colony stimulating factor 3 (CSF3; G-CSF) treated mice, lipopolysaccharide treated mice, and multiple genetically-engineered mouse cancer models. In these models, neutrophil populations alter their cell surface adhesion and chemokine receptor expression, compared with mature neutrophils. Interestingly, immature neutrophils also differ in their capacity to generate reactive oxygen species production with and without stimulation. We also measured the differential capacity of these populations to suppress cytotoxic lymphoid cell activity. Overall, circulating immature neutrophils are present in models of cancer, severe infection and emergency granulopoiesis where they display different functional capacity compared to mature neutrophils.

WS.B2.03.06

Epithelial damage and tissue γδ T cells promote a unique tumour-protective IgE response

G. Crawford[1], *R. Castro Seoane*[1], *S. Ward*[1], *M. Hayes*[1], *M. Haniffa*[2], *D. Dunn-Walters*[3], *J. Strid*[1];

[1]*Imperial College London, London, United Kingdom,* [2]*Newcastle University, Newcastle, United Kingdom,* [3]*University of Surrey, Guildford, United Kingdom.*

IgE is an ancient and conserved immunoglobulin isotype with potent immune function. Nevertheless, the regulation of IgE responses remains enigmatic and evidence for a role of IgE in host defense is limited. We have previously described that IgE is strongly induced through the skin as part of the lymphoid stress-surveillance (LSS) response. LSS refers to the capacity of resident γδTCR$^+$ intraepithelial lymphocytes (IEL) to directly sense epithelial cell dysregulation and initiate a restorative response. Here we explore the afferent pathways leading to IgE following skin exposure to environmental xenobiotics and the role of this endogenous IgE in controlling spontaneous tumour growth. We demonstrate the development of a potent autoreactive IgE response following topical exposure to the common environmental xenobiotic and carcinogen, 7,12-Dimethylbenz(a)anthracene. Further we show how tissue γδTCR$^+$ IELs are uniquely initiating and regulating the IgE, but not IgG1, response. High-throughput antibody sequencing reveals that γδ T cells shape the IgE repertoire by supporting specific VDJ rearrangements with unique CDRH3 characteristics. This endogenous IgE response, via the FcεRI, protects against epithelial skin carcinogenesis and *FceR1a* expression in human squamous cell carcinoma correlates inversely with poor disease outcome. Thus, LSS promotes a unique IgE response, which is part of an early host defense mechanism providing protection against cancer. Our data provides experimental support for the 'toxin hypothesis', suggesting IgE as an intentional host defense mechanism against non-infectious cell-damaging environmental xenobiotics and propose a cooperative role for T and B cell immune surveillance in epithelial tissues.

WS.B3.01 Molecular regulation of T cell responses

WS.B3.01.01

Resident CD4+ T cells in secondary lymphoid organs

A. C. Kaminski[1], *O. Pabst*[1], *M. Ugur*[2];

[1]*Molecular medicine, Aachen, Germany,* [2]*Department of Microbiology and Immunology, Melbourne, Australia.*

Adaptive immunity relies on controlled lymphocyte migration and proper lymphocytes positioning. We and others established in vivo cell tracking based on photoconvertible proteins. Photoconversion enables the labelling of immune cells in situ and is thus particularly suited for studying cell egress and migration. To enable long-term tracking of photoconverted cells, we established a histone-fused photoconvertible Dendra2 protein. Retroviral overexpression of histone-fused Dendra2 and in vivo cell tracking experiments allowed us to identify a population of lymphoid node (LN) resident CD4+ T cells. In this project we analyse the molecular and functional characteristics of LN resident CD4+ T cells. We use a novel transgenic mouse with stable expression of histone-fused Dendra2 in immune cells. Naïve CD4+ T cells typically leave LNs after 8-12 hours. Recently activated T effector cells stay in LNs for approximately 3 days. We have identified another migratory pattern: resident CD4+ T cells remain in peripheral LNs for at least up to 28 days. Resident CD4+ T cells constitute 20-50% of all effector/memory CD4+ T cells, indicating a major population of effector/memory CD4+ T cells with hitherto unappreciated migratory behaviour. Our data indicate that generation of resident CD4+ T cells requires strong immune stimulation and sustained availability of antigen. Resident CD4+ T cells were maintained in a model of induced T cell receptor deficiency. This indicates that CD4+ T cells can remain resident in LN independently of continuous T cell receptor triggering. At present, we further characterise the mechanisms to generate and maintain resident CD4+ T cells.

WS.B3.01.02

Production of extracellular adenosine by CD73$^+$ dendritic cells is crucial for induction of T cell hyporesponsiveness

C. Silva-Vilches, *K. Mahnke;*
University Hospital Heidelberg, Heidelberg, Germany.

Dendritic cells (DCs) express the ecto-5'-nucleotidase CD73 that generates immunosuppressive Adenosine (Ado) by dephosphorylation of extracellular Adenosine-monophosphate. To investigate whether Ado that is produced by CD73$^+$ DCs affects induction of tolerance in contact hypersensitivity (CHS) reactions, C57BL/6 wildtype (WT) and CD73$^{-/-}$ animals were tested in a 2,4-dinitrofluorobenzene (DNFB) induced-CHS model. In this model pre-treatment with 2,4-dinitrothiocyanobenzene (DNTB) induces tolerance to DNFB. We demonstrate that treatment with DNTB induced tolerance to DNFB only in WT but not in CD73$^{-/-}$ mice. Analysis of DCs that migrated from skin to draining lymph nodes (smDCs) in WT mice revealed increased expression of CD73 after application of DNTB and a lower expression of activation markers (CD80 and CD86) compared to CD73$^{-/-}$ smDCs, accompanied by elevated concentrations of extracellular Ado within the LN tissue. Also, markers of T cell anergy, namely early growth response protein-2 (EGR2) and N-Myc downstream regulated protein-1 (NDRG1) were upregulated in LN T cells of DNTB-treated WT mice. Moreover, those T cells from WT animals exhibited less proliferation, less activation and less cytokines production than T cells from CD73$^{-/-}$ mice upon *ex vivo* re-stimulation. Similar effects were observed *in vitro*.
I.e., Ado-producing WT DCs, but not CD73$^{-/-}$ DCs rendered T cells hyporeactive, decreased their T cell costimulatory signaling and induced upregulation of EGR2 and NDRG1. Thus, these data demonstrate that expression of CD73 by DCs, which triggers elevated levels of extracellular Ado, is a crucial mechanism for the induction of anergic T cells and tolerance.
Funded by SFB/TR156

WS.B3.01.03

EGFR-HIF1α signaling positively regulates the development of Th9 cells

S. Roy, *Z. Rizvi, A. Awasthi;*
Translational Health Science and Technology Institute (THSTI), Faridabad, India.

Epidermal growth factor receptor (EGFR) is crucial for the proliferation and differentiation of immune cells and is important for the development of tumors. EGFR has been shown to be expressed on T cells and play a role in T cell differentiation and regulation. The role of EGFR and its ligands in IL-9-producing Th9 cells has not been identified yet. Th9 cells play a critical role in inducing anti-tumour immunity, allergic inflammation as well as in autoimmunity. To understand the transcriptional network of Th9 cells using RNASeq analysis, we identified a strong upregulation of EGFR expression in Th9 cells. We have identified that the expression of EGFR is required for the induction of IL-9 in Th9 cells, as EGFR specific inhibitor, Gefitinib, suppressed the development of Th9 cells. Further analysis of EGFR pathways in Th9 cells revealed EGFR activation upregulates hypoxia-inducible factor 1α (HIF1α) and inducible nitric oxide synthase (iNOS) in Th9 cells. The increased expression of HIF1α and iNOS increases the production of amphiregulin, an EGFR ligand in Th9 cells, which acts in a feed-forward loop further enhancing Th9 cells differentiation. Mechanistically, we have identified that HIF1α binds and transactivates iNOS and IL-9 in Th9 cells. Furthermore, inhibition of EGFR and HIF1α pathway suppressed IL-9 production in both mouse and human Th9 cells, and substantially promoted the tumor development. Our findings thus identify a critical role of EGFR-HIF1α module in the development and functions of Th9 cells which can be targeted for successful immunotherapy.

WS.B3.01.04

Post-transcriptional regulation of cytokine production in T cells by RNA binding proteins

B. Popovic[1], *F. Şalerno*[1], *S. Engels*[1], *F. van Alphen*[2], *A. Guislain*[1], *B. P. Nicolet*[1], *M. C. Wolkers*[1];
[1]*Sanquin Research/Landsteiner Laboratory, Amsterdam, Netherlands,* [2]*Sanquin Research, Amsterdam, Netherlands.*

Effective cytotoxic CD8+ T cell responses depend on the concerted production of inflammatory cytokines and cytotoxic molecules. The co-production of IFNγ, TNFα and IL-2 is a hallmark of the most potent effector cells. We recently showed that post-transcriptional regulatory mechanisms are key to fine-tune cytokine production. Both the translational rate and stabilization of cytokine mRNA was a determinant of production levels. Intriguingly, all three cytokines used private regulatory mechanisms for their production: whereas the production of TNFα almost exclusively depended on the translational rate, IL-2 primarily depended on *de novo* transcription, and mRNA stabilization was only used to amplify the production rate. IFNγ used all three regulatory mechanisms for optimal production, i.e. *de novo* transcription, translation efficiency and mRNA stabilization. Here we set out to identify which factors drive this individual regulation of cytokine mRNA, with a specific focus on regulatory RNA binding proteins (RBPs). To this end, we employed streptavidin-binding RNA aptamers containing the 3'Untranslated Region (3'UTR) of the three mRNAs to identify RBPs binding to the individual 3'UTR. Using lysates from *in vitro* expanded, resting primary human T cells, we identified differential binding of RBPs by mass spectrometry analysis to each individual cytokine 3'UTR. We currently investigate the mode of action of the identified RBPs to fine-tune cytokine production. Unraveling the post-transcriptional mechanisms that define the cytokine production in T cells should yield valuable biological insights that will help driving effector responses against pathogens and malignant cells.

WS.B3.01.05

Lysosome-related organelle tethering controls directionally distinct cytokine transport in T cells

B. Qu*, Y. Zhou, R. Zhao, E. C. Schwarz, R. Akbar, V. Pattu, V. Helms, H. Rieger;*
Saarland University, Homburg, Germany.

Cytokines are small proteins playing a key role in orchestrating activation and responses of immune cells. It is of markedly importance to target cytokines to the desired destination. In $CD4^+$ T cells, in particular, cytokines are delivered in two distinct modes: either exclusively to the immunological synapse (IS) (eg. IL-2) or transported multi-directionally (eg. TNFα). However, the molecular mechanisms responsible for these distinct transport patterns remain elusive. Here we show that in primary human $CD4^+$ T cells both $TNFα^+$ and $IL\text{-}2^+$ vesicles tether with lysosome-related organelles (LROs), mediated by the SNARE protein Vti1b. Only LRO-tethered but not untethered cytokine vesicles are preferentially transported to the desired secretion sites, namely LRO-tethered $IL\text{-}2^+$ to the IS and LRO-tethered $TNFα^+$ multi-directionally. LRO tethering enhances the recruitment of kinesin and dynein to $TNFα^+$ vesicles, favoring the transport to both directions along microtubules. In contrast, the recruitment of dynein but not kinesin is selectively enhanced in LRO-tethered $IL\text{-}2^+$ vesicles, promoting minus-end transport to the MTOC, which localizes at the IS. Our findings suggest that LRO tethering serves as a novel mechanism to regulate directionally distinct cytokine transport, especially in $CD4^+$ T cells.

WS.B3.01.06

Targeting E3 ubiquitin ligase receptor cereblon to stimulate Myc-driven metabolismin CD8+ T cells

R. Hesterberg, A. A. Akuffo, M. S. Beatty, W. E. Goodheart, M. Fernandez, J. L. Cleveland, ***P. K. Epling-Burnette****;*
Moffitt Cancer Center, Tampa, United States.

Derivatives of thalidomide known as immunomodulatory drugs (IMiD) bind to and elicit anti-cancer and immune modulating activity by binding to the E3 ubiquitin ligase substrate receptor cereblon (CRBN). While IMiDs potentiate T cell effector functions in healthy and immunosuppressed cancer patients, the physiological roles of CRBN in T cells is undefined. To understand the immunological role of CRBN in T cells, we made use of a germline *Crbn* knockout mouse ($Crbn^{-/-}$) and created OT1;$Crbn^{-/-}$ mice as well as LckCre;$Crbn^{fl/fl}$ mice. T cells derived from $Crbn^{-/-}$ mice manifest higher rates of proliferation and cytokine production and an elevated bioenergetics profile, with supraphysiological levels of polyamines secondary to enhanced glucose and amino acid transport and increased expression of metabolic enzymes including ornithine decarboxylase related to stabilization of c-Myc.
To determine if these changes occur in the presence of IMiD treatment, we examined Myc expression and metabolic rates after T cell activation and found that both IMiDs and Crbn deficiency increased and sustained the expression of the master metabolic regulator Myc. While *Crbn* deficient T cell can exacerbate graft-versus-host (GVHD) disease *in vivo*, they also endow tumor infiltrating lymphocytes with superior anti-tumor reactivity following adoptive transfer and after T cell-specific deletion of *Crbn*. Therefore, CRBN represents a druggable target that has a profound effect on sustaining the immunometabolism of CD8+ T cells.

WS.B3.02 T cell mediated immune regulation in tumors

WS.B3.02.01

HDAC7 controls $CD8^+$T cell dependent anti-tumor immunity

C. Yerinde[1,2,3], *J. Keye*[1,2], *F. Schmidt*[1,2], *B. Siegmund*[1], *R. Glauben*[1], *C. Weidinger*[1,4];
[1]*Charité - Universitätsmedizin Berlin, Medical Department, Division of Gastroenterology, Infectiology and Rheumatology, Campus Benjamin Franklin, Berlin, Germany,* [2]*Freie Universität Berlin, Department of Biology, Chemistry and Pharmacy, Berlin, Germany,* [3]*Berlin School of Integrative Oncology (BSIO) MKFZ, Berlin, Germany,* [4]*Berlin Institute of Health (BIH), Clinician Scientist Program, Berlin, Germany.*

Class II Histone deacetylases (HDAC) were shown to orchestrate T cell-dependent immune responses via the epigenetic control of genes and via the post-translational modification of cytoplasmic and nuclear proteins. However, the contribution of single HDAC family members to T cell differentiation and function remain elusive. To elucidate the role of histone deacetylase 7 (HDAC7) in T cells, we assessed the immune cell composition of $Hdac7^{flox/flox}$*Cd4-Cre* mice by mass cytometry under steady state conditions using a panel of 31 classifying-, differentiation- and activation- markers, which revealed a highly pre-activated phenotype within the $CD8^+$ T cell compartment. By using Seahorse analysis and RNA sequencing, we observed that deletion of HDAC7 reduced the cellular expansion of $CD8^+$ T cells, due to the transcriptional deregulation of metabolism- and apoptosis-regulating genes resulting in an impaired glycolytic capacity and apoptosis of $CD8^+$ T cells. Furthermore, HDAC7-deficient $CD8^+$ T cells harbored impaired production of IFNγ, which could be linked to reduced activation levels of Store-operated Calcium Entry (SOCE) signaling. Importantly, $Hdac7^{flox/fox}$*Cd4-Cre* mice formed significantly bigger tumors when injected with the syngenic lymphoma cell line EG-7, as $Hdac7^{fl/fl}$*Cd4-Cre* mice harbored significantly lower numbers of tumor-infiltrating $CD8^+$T cells in comparison to wild type littermates. Taken together our data reveal that HDAC7 might serve as a key regulator of T cell mediated anti-tumor immunity via controlling the metabolism and calcium homeostasis of $CD8^+$ T cells.

WS.B3.02.02

Deciphering the molecular pathways underlying oncogenic IL-7R signaling in T-cell acute lymphoblastic leukemia

A. Murcia Ceballos[1], *P. Fuentes Villarejo*[1], *S. González García*[1], *M. García Peydró*[1], *J. Alcain*[1], *E. García Martínez*[1], *A. A. Ferrando*[2], *M. Toribio*[1];
[1]*Centro de Biología Molecular "Severo Ochoa", Madrid, Spain,* [2]*Institute for Cancer Genetics, New York, United States.*

Introduction: Interleukin-7 plays a crucial role in normal T and B-cell development and also contributes to proliferation in acute lymphoblastic leukemia (ALL), the most common pediatric cancer. Recently, *IL7R* mutations have been described in 10% of T and B-ALL cases, and mutational activation of IL-7R was suggested to be involved in T-cell leukemogenesis. We thus sought to investigate intracellular pathways underlying oncogenic IL-7R signaling in T-ALL, with the final aim of delineating specific therapeutic strategies.
Methods: We investigated the impact of a novel gain-of-function mutation located in *IL7R* exon6 identified in T-ALL patient, by expression in cytokine-dependent and independent cell lines, and primary human early thymic precursors and T-ALL cells. Fetal thymus organotypic cultures (FTOC) allowed us to assess the step-wise oncogenic impact of mutant IL-7R in T-cell development. Moreover, biochemical approaches were performed in combination with *in vitro* cell proliferation and *in vivo* tumor progression assays of cells expressing mutant IL-7R.
Results: We show that mutant IL-7R promoted the selective expansion and accumulation of primary human pre-T cells in FTOC, and the constitutive proliferation and survival of cytokine-dependent cells *in vitro*. More importantly, it also enhanced tumor progression of cell lines and primary T-ALL cells *in vivo*. This functional impact was associated with constitutive JAK-STAT, MAPK and mTORC1 activation and c-myc expression, independently of PI3K-Akt activation. Possible pathways underlying mutant IL-7R-dependent constitutive mTORC1 activation will be discussed.
Conclusions: The identification of mTORC1 activation through an Akt-independent pathway, points to novel molecules as promising and unexplored therapeutic targets for T-ALL.

WS.B3.02.03

Epithelial signals promote local effector Foxp3$^+$ regulatory T cell accumulation in the gut

*A. Bremser[1], H. Meinicke[2], **A. Izcue**[3];*
[1]Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany, [2]Freiburg University Hospital, Freiburg, Germany, [3]Institute of Molecular Medicine, Aachen, Germany.

Tumors are often accompanied by a local accumulation of CD4$^+$ Foxp3$^+$ regulatory T cells (Treg) that can inhibit anti-tumoral responses. However, the pathways leading to local Treg accumulation and whether accumulating Treg represent a specific Treg population remain to be investigated. Using genetically modified mice we have found that cadherin switch and increased β-catenin in intestinal epithelial cells, two features associated with malignant transformation, lead to a striking increase in the frequency and numbers of the KLRG1$^+$ GATA3$^+$ Treg subset. Although KLRG1 is a ligand for E-cadherin and KLRG1 expression can reduce Treg accumulation in the gut, ablation of KLRG1 on Treg did not lead to an increase in the gut Treg populations. Rather, both E-cadherin ablation and increased β-catenin signals resulted in higher levels of interleukin-33 in epithelial cells. We found that IL-33 preferentially expands KLRG1$^+$ GATA3$^+$ Treg cells in vitro, suggesting that IL-33 could link alterations in epithelial cells with a local increase in Treg. Intestinal tumors often present reduced E-cadherin expression, enhanced β-catenin signaling and increased IL-33. Analysis of intestinal tumors from APC$^{min/+}$ mice showed that local Treg accumulation in this model was exclusively due to an increase in KLRG1$^+$ GATA3$^+$ Treg cells. Our data identify a novel axis through which epithelial cells control local Treg cell subsets, an axis which may be activated during intestinal tumorigenesis.

WS.B3.02.04

Intravital signaling visualization reveals that transient productive interactions between T regulatory cells and classical DC support cancer immune tolerance

***F. Marangoni**[1], E. Carrizosa[1], V. Mani[1], M. Thelen[2], J. Prüssmann[1], T. R. Mempel[1];*
[1]Massachusetts General Hospital - Harvard Medical School, Charlestown, United States, [2]Uniklinik Köln, Köln, Germany.

The recognition of cognate antigen within tumors is necessary for CD4$^+$Foxp3$^+$ T regulatory cells (Treg) to enforce local immunological tolerance (Bauer JCI 2014). However, how cellular interactions, tightly controlled in space and time, regulate this process *in vivo* is unknown. We visualized Treg activation using an NFAT-GFP construct whose nuclear localization reports the activation of TCR-calcineurin-NFAT signaling (Marangoni Immunity 2013). NFAT-GFP-transduced Treg were transferred in mice with red-fluorescent CD11c cells, and imaged within implanted tumors by multiphoton microscopy. We found that ~30% of polyclonal Treg, but no conventional CD4$^+$ T cells (Tconv), were activated in tumors. This was due to differences in the TCR repertoire because Treg and Tconv with an identical hemagglutinin (HA)-specific TCR were activated in HA-expressing tumors. Notably, while HA-Tconv:CD11c interactions were stable, HA-Treg:CD11c contacts were short-lived.
This was not due to characteristics intrinsic to Treg but to their capacity to progressively modulate antigen presentation by APC, because HA-Treg were capable of stable interactions early after transfer but destabilized HA-Tconv:CD11c interactions over time. We studied the meaning of Treg:CD11c interactions by inducing Treg-specific deletion of calcineurin B (CnB), which reduced tumor-associated Treg numbers, lowered the expression of ICOS and CTLA4, and delayed tumor growth. Deletion experiments identified zbtb46-dependent classical DC as the APC activating tumor-associated Treg. We thus conducted the first imaging-based investigation of Treg activation demonstrating that short-lived productive interactions with classical DC, which may be detrimental for conventional T cell function (Marangoni Immunity 2013), conversely suffice to maintain Treg numbers and immunosuppressive capacity in tumors.

WS.B3.02.05

The CD58-CD2 axis controls killing of leukemic B cells by cytotoxic T lymphocytes

***V. Zurli**[1], T. Montecchi[1], G. Wimmer[1], O. Acuto[2], C. Baldari[1], A. Kabanova[1];*
[1]University of Siena, Siena, Italy, [2]University of Oxford, Oxford, United Kingdom.

Introduction: Cytotoxic T lymphocytes (CTLs) play a key role in the immune defence against cancer. To exert their vital function, CTLs polarize and focally release lytic granules at the immune synapse formed with their targets. We previously reported that leukemic B cells can resist CTL killing by establishing dysfunctional synapses characterized by non-polarized granule release. In the current work we address the mechanisms controlling lytic granule polarization in human CTLs.
Methods: Firstly, we identified the factor responsible for the formation of dysfunctional synapses by applying comparative proteomics analysis of surface proteins to a panel of B cells, either susceptible or resistant to CTL lysis. Then we used CRISPR/Cas9 technology, functional assays, and phospho-proteomics to characterize molecules involved in the lytic granule movement in CTLs.
Results: We identified four co-stimulatory molecules highly expressed on susceptible B cells: CD58, CD30, SLAMF-1 and SLAMF-7. Among these, only CD58, through the interaction with its receptor CD2 on CTLs, was able to promote granule polarization towards the synapse. Notably, we found that CTL costimulation through CD2 was of crucial importance for B cell killing. We are currently applying a phospho-proteomics approach to elucidate the CD2 signaling network implicated in granule polarization.
Conclusions: Considering that loss of CD58 expression on B-cell tumours has been described as the mechanism of cancer progression, our results both highlight new key aspects of CTL biology and are expected to be valuable for the development of focused therapies counteracting cancer immune evasion.
Grants: ITT (C.T.B.), AIRC TRIDEO (A.K.), AIRC fellowship (V.Z.)

WS.B3.02.06

Functional anti-TIGIT antibodies modulate T cell responses *in vivo*

*K. O. Dixon[1], **M. Schorer**[2], J. Nevin[1], Y. Etminan[1], Z. Amoozgar[3], T. Kondo[1], S. Kurtulus[1], N. Kassam[1], R. A. Sobel[4], D. Fukumura[3], R. K. Jain[3], A. C. Anderson[1], V. K. Kuchroo[1], N. Joller[2];*
[1]Evergrande Center for Immunologic Diseases and Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, United States, [2]Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland, [3]Edwin L. Steele Laboratories, Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, United States, [4]Palo Alto Veteran's Administration Health Care, Department of Pathology, Stanford University School of Medicine,, Stanford, United States.

Introduction: The selective engagement of inhibitory receptors plays an important role in maintaining immune homeostasis and their divergent expression gives rise to a broad range of pathologies such as autoimmunity, cancer and chronic infections. Blocking antibodies directed against co-inhibitory receptors such as PD-1 and CTLA-4 showed great efficacy as anti-cancer drugs in the clinics because of their potential to restore T cell responses *in vivo*. TIGIT is a novel co-inhibitory receptor that has recently gained attention as a potential regulator of T cell exhaustion in the context of cancer as well as in ameliorating autoimmune disorders. Materials and Methods: In order to study the immune modulatory properties of TIGIT, we generated a panel of functional anti-TIGIT antibody clones using hybridoma cultures and tested them in both autoimmune and cancer models. Results: We found that the administration of the agonistic anti-TIGIT antibody ameliorated autoimmune disease severity in EAE, whereas administration of the blocking anti-TIGIT antibody in combination with anti-PD-1 blockade showed a synergic anti-tumor effect in models of colon carcinoma and glioblastoma. Conclusions: Collectively, our data demonstrates that TIGIT modulation can be used to effectively regulate T cell responses and disease outcome *in vivo* and provides further insight for the development of novel therapeutic approaches.

WS.B3.03 T cell responses in health and disease

WS.B3.03.01

Loss of NIK in thymic epithelial cells leads to fatal autoimmunity through aberrant Treg development

***C. Haftmann**[1], F. Mair[1], E. Terskikh[1], M. Spalinger[2], B. P. Leung[3], A. Waisman[4], B. Becher[1];*
[1]Inst. of Experimental Immunology, Zürich, Switzerland, [2]Universitätsspital, Klinik für Gastroenterologie und Hepatologie, Zürich, Switzerland, [3]University of Southern California, Department of Physiology & Biophysics, Zürich, Switzerland, [4]Johannes Gutenberg University Mainz, Inst. for Molecular Medicine, Mainz, Germany.

Medullary thymic epithelial cells (mTECs) mediate central T cell tolerance through negative selection. mTecs are, however, also critical for the formation of peripheral tolerance by instructing the development of nTregs. Which of these two functions dominate is a matter of some debate. We have generated a mouse model in which the development and function of mTECs are compromised by conditional ablation of the NF-kB inducing kinase (NIK, encoded by *Map3k14*) restricted to the TEC compartment (mTEC-NIK). In contrast to germ-line deficient *NIK$^{-/-}$* mice, which develop largely normally and show no overt pathology, mTEK-NIK mice rapidly developed severe and fatal multi-organ autoimmunity shortly after birth, characterized by infiltration of effector T cells in various organs. The observed autoimmunity was clearly T cell mediated, as the survival of the mice could be significantly improved upon antibody mediated T cell depletion. However, whereas there was no obvious indication of augmented auto-reactivity through the loss of negative selection, Tregs, although emerging from the thymus, were dysfunctional as indicated by high-parametric single cell analysis of Treg markers and *in vivo* suppression assays. Conversely, adoptive transfer of normal Tregs prevented autoimmunity. Most strikingly, in thymic co-transplantation, Tregs primed by wildtype thymi were able to prevent autoimmune pathology by mTEC-NIK educated T cells. We thus postulate that Treg induction rather than negative selection of autoreactive T cell clones is the superior duty of mTECs in thymic T cell education. The author received a fellowship of the German Research Council (DFG).

WS.B3.03.02

A Timer for analyzing temporally dynamic changes in transcription during T cell differentiation in vivo

*D. Bending[1], P. Prieto Martín[1], A. Paduraru[1], C. Ducker[1], E. Marzaganov[1], M. Laviron[1], T. Crompton[2], **M. Ono**[1];*
[1]Department of Life Sciences, London, United Kingdom, [2]University College London, London, United Kingdom.

Background: Understanding the mechanisms of cellular differentiation is challenging because differentiation is initiated by signaling pathways that drive temporally dynamic processes, which are difficult to analyse in vivo. **Results**: We establish a new Tool, Timer-of-cell-kinetics-and-activity (Tocky [toki], time in Japanese). Tocky uses the Fluorescent Timer protein, which spontaneously shifts its emission spectrum from blue-to-red, in combination with computer algorithms to reveal the dynamics of differentiation in vivo. Using a transcriptional target of T cell receptor (TCR)-signaling, we establish *Nr4a3*-Tocky to follow downstream effects of TCR signaling. Using *Nr4a3*-Tocky, we determined the temporal sequence of events during regulatory T cell (Treg) differentiation, identified the major precursor population of Treg, and showed that persistent TCR signals leads to the initiation of *Foxp3* transcription and Treg generation. Interestingly, in the periphery, *Nr4a3*-Tocky showed that self-reactive T cells, which include Treg and memory-phenotype T cells, received spontaneous infrequent TCR signals. Furthermore, using the murine model of Multiple Sclerosis, myelin-specific T cells at the site of autoimmune inflammation also showed persistent TCR signalling, which *Nr4a3*-Tocky distinguished from infrequent TCR signals in self-reactive T cells. In addition, by generating *Foxp3*-Tocky, we establish that the Tocky approach can be applied to another gene as well. By analysing differentiating Treg using *Foxp3*-Tocky, we showed a progressive demethylation of the *Foxp3* gene across time, demonstrating that the active demethylation process occurred in T cells with sustained *Foxp3* transcription. **Conclusion**: Tocky is a tool for cell biologists and immunologists to address previously inaccessible questions by directly revealing dynamic processes in vivo. **Funder**: Biotechnology and Biological Sciences Research Council

WS.B3.03.03

A temporally dynamic Foxp3 autoregulatory transcriptional circuit controls the effector Treg programme

***D. Bending**[1], A. Paduraru[2], C. Ducker[2], P. Prieto Martin[2], T. Crompton[3], M. Ono[2];*
[1]University of Birmingham, Birmingham, United Kingdom, [2]Imperial College London, London, United Kingdom, [3]University College London, London, United Kingdom.

Foxp3$^+$ Regulatory T-cells (Treg) are negative regulators of the immune response, and upon activation, Treg can suppress the activities of other T cells as effector Treg. Whilst thymic Treg development has been extensively characterised, it remains largely unknown whether and how *Foxp3* transcription is induced and regulated in the periphery during T-cell responses. In addition, it is poorly known what controls the differentiation of Treg into effector Treg, which are Treg with enhanced expression of potent immunoregulatory molecules, such as IL-10. Here, using our state of the art *Foxp3-Timer of cell kinetics and activity* (Tocky) mice, which capture both quasi real-time and historical *Foxp3* expression, we show that both the generation of new *Foxp3* expressors and the rate of *Foxp3* transcription are dramatically increased during inflammation. These persistent dynamics of *Foxp3* transcription drive the effector-Treg programme and are dependent on a Foxp3 protein-dependent autoregulatory transcriptional circuit, which simultaneously sustains *Foxp3* transcription whilst repressing IL-2 expression. Persistent *Foxp3* transcriptional activity controls the expression of coinhibitory molecules, including CTLA-4 and effector-Treg signature genes.
Using RNA-seq, we re-classify key Treg-associated surface proteins based on their relationship to the temporal dynamics of *Foxp3* transcription. Furthermore, we show proof-of-principle for the manipulation of Foxp3$^+$ T-cell dynamics by immunotherapy: new Foxp3 flux is promoted by anti-TNFRII antibody, and high frequency *Foxp3* transcribers are targeted by anti-OX40 antibody. Collectively, our study dissects time-dependent mechanisms behind Foxp3-driven T-cell regulation and establishes the *Foxp3*-Tocky system as a tool to investigate the mechanisms behind Treg-targeting immunotherapies.

WS.B3.03.04

N4BP1 and TNIP1 control MHC-1 display on neuroblastoma tumors

*L. Spel[1], J. Nieuwenhuis[2], R. Haarsma[1], E. Stickel[2], O. Bleijerveld[2], M. Altelaar[3], j. Boelens[1], T. Brummelkamp[2], S. Nierkens[1], **M. Boes**[1];*
[1]UMC-Utrecht, Utrecht, Netherlands, [2]Netherlands Cancer Institute, Amsterdam, Netherlands, [3]Utrecht University, Utrecht, Netherlands.

Neuroblastoma is the second most common tumor in children. The cause of neuroblastoma is thought to originate from derailed development of embryonic neural crest cells and is accompanied by low MHC-1 expression and suppression of the NF-κB transcription factor. Here, we addressed MHC-1 gene regulation in neuroblastoma, with ultimate goal to enhance its immunogenic potential for therapeutic T-cell targeting. Using a genome-wide CRISPR screen, we identified N4BP1 and TNIP1 as inhibitory factors of NF-κB-mediated MHC-1 expression in neuroblastoma. In support for a clinical relevance of these NF-κB-inhibiting factors, advanced stage neuroblastoma patients who express high levels of TNIP1 and N4BP1 have worse overall survival. Depletion of N4BP1 or TNIP1 indeed increased NF-κB and MHC-1 expression, and stimulated recognition by antigen-specific CD8+ T-cells. We confirmed that TNIP1 inhibits canonical NF-κB member RelA by preventing activation of the RelA/p50 NF-κB dimer. Furthermore, we show that N4BP1 inhibits both canonical and non-canonical NF-κB through binding of deubiquitinating enzyme CEZANNE, resulting in stabilization of TRAF3 and degradation of NF-κB-initiating kinase NIK. Thus, N4BP1/CEZANNE or TNIP1 are candidate immunotherapy targets in neuroblastoma tumors that should lift NF-κB suppression, and thereby trigger increased peptide/MHC1-mediated tumor reactivity to enhance therapeutic T-cell targeting.

WS.B3.03.05

T cell-induced tumor vulnerability discovery in anIFNg-independent genomic landscape

*D. Peeper, **D. Vredevoogd**;*
The Netherlands Cancer Institute, Amsterdam, Netherlands.

New clinical opportunities are needed to increase immunotherapy (IT) benefit. Whereas IFNγ-pathway mutations cause IT resistance, we find that IFNγ receptor-deficient tumors remain remarkably susceptible to T cell elimination. By integrating transcriptomics, proteomics and genome-wide CRISPR-Cas9 screening with clinical data, we defined the IFNγ-independent tumor landscape and uncovered a new immunotherapeutic opportunity. Ablation of TRAF2 enhanced T-cell elimination of both melanoma and lung cancer cells by redirecting TNF signaling to favor RIPK1-dependent apoptosis. TRAF2 loss greatly enhanced the therapeutic potential of pharmacological inhibition of its interaction partner cIAP. Corroborating these observations clinically, we identified recurring TRAF2 mutations that reduced T-cell sensitivity of tumor cells. Furthermore, both IT response duration and overall survival were lower for tumors that had acquired TNF pathway mutations on IT, suggesting immune-editing by TNF pressure. These results functionally annotate the tumor genome landscape of IFNγ-independent T cell cytotoxicity, and merit clinical exploration of TRAF2 for cancer immunotherapy.

WS.B3.03.06

NKG2A blockade potentiates CD8$^+$ T-cell immunity induced by cancer vaccines

***T. van Hall**[1], N. van Montfoort[1], L. Borst[1], M. J. Korrer[2], Y. Kim[2], S. Santegoets[1], M. Welters[1], P. Andre[3], S. Piersma[4], S. H. van der Burg[1];*
[1]Leiden University Medical Center, Leiden, Netherlands, [2]Vanderbilt University, Nashville, United States, [3]Innate Pharma, Marseille, France, [4]University of Washington, St Louis, United States.

Cancer vaccination has shown thus far limited clinical efficacy due to multiple suppressive factors in the tumour environment. We now demonstrate that the inhibitory receptor NKG2A constitutes an adaptive resistance mechanism during cancer vaccination by interaction with HLA-E on tumour cells. This immune receptor was preferentially found on tumour-infiltrating natural killer cells and CD8$^+$, but not CD4$^+$, T cells. Expression on CD8$^+$ T cells was found on CD103$^+$ tumour-infiltrating cells and only partly overlapped with other checkpoint receptors. Particularly high frequencies of NKG2A-expressing lymphocytes were detected in tumours with an immune-reactive profile and could be induced by therapeutic cancer vaccination. To examine if NKG2A represented an adaptive resistance mechanism during cancer vaccination, we blocked the receptor with a therapeutic antibody and performed genetic knockdown experiments for its ligand Qa-1, the conserved ortholog of HLA-E. In four mouse tumour models, the modest effect of therapeutic vaccines was greatly potentiated by disruption of the NKG2A/Qa-1 axis. NKG2A blockade operated through CD8$^+$ T cells and was even effective in a mouse model refractory to anti-PD-1 therapy. These findings indicate that NKG2A-blocking antibodies might improve clinical responses to therapeutic cancer vaccines.

WS.B4.01 Molecular control of T cell activation and exhaustion

WS.B4.01.01

PTPN22-deficient CD8 T cells resist TGFb suppression and mediate improved anti-tumour responses

*R. J. Brownlie[1], C. Garcia[2], M. Ravasz[2], D. Zehn[3], R. Zamoyska[2], **R. J. Salmond**[1];*
[1]Universty of Leeds, Leeds, United Kingdom, [2]Universty of Edinburgh, Edinburgh, United Kingdom, [3]Technical University Munich, Munich, Germany.

T cell adoptive therapy has the potential to treat human malignancies. Nonetheless, the poor immunogenicity of tumours and a strongly suppressive tumour microenvironment, characterized by high levels of inhibitory cytokines such as TGFb, limit the efficacy of this approach. We have previously shown that T cells lacking inhibitory phosphatase PTPN22 have enhanced responses to weak antigens (1), which may be of benefit in a tumour setting. We now show that PTPN22-deficient CD8+ T cells also have a substantially reduced susceptibility to TGFb inhibition. Subsequently, tumour-reactive PTPN22-/- CD8 T cells are superior in their ability to clear weakly immunogenic TGFb-secreting tumours in vivo. Mechanistically, in the absence of PTPN22, TCR-induced NFAT activation and IL-2 secretion is enhanced, which enables T cells to overcome the anti-proliferative effects of TGFb (2). Furthermore, we present data showing that PTPN22 also influences anti-tumour T cell memory.
1) Salmond et al, Nat. Immunol. 2014, 15:875
2) Brownlie et al, Nat. Comms. 2017, 8:1343

WS.B4.01.02

Heterochromatin silencing of stemness during CD8⁺ T cell fate commitment

L. Pace[1,2,3], C. Goudot[4,3], E. Zueva[2,3], P. Gueguen[2,3], N. Burgdorf[2,3], J. Waterfall[5,3], J. Quivy[6,3], G. Almouzni[6,3], S. Amigorena[2,3];
[1]Italian Institute Genomic Medicine (IIGM), Turin, Italy, [2]U932, Curie Institute, Paris, France, [3]PSL Research University, Paris, France, [4]U932, Institute Curie, Paris, France, [5]U830, Curie Institute, Paris, France, [6]UMR3664, Curie Institute, Paris, France.

Following activation by antigens, naïve CD8⁺ T lymphocytes establish specific heritable gene expression programs that define the progression to long-lasting memory or to short-lived effector cells. While lineage specification in T cells is critical for protection, the impact of epigenetic silencing on T lymphocyte differentiation is still incompletely understood. Here, we explore the role of heterochromatin-mediated gene expression silencing by Suv39h1, a histone H3 lysine 9 methyltransferases that plays a critical, evolutionary conserved, role in heterochromatin structure and dynamics. We show that in murine CD8⁺ T cells activated after *Listeria monocytogenes* infection *in vivo*, Suv39h1-dependent H3K9me3 deposition controls the expression of a set of stem cell-related/memory genes. Single-cell RNA sequencing analysis reveals that the silencing in stem/memory genes selectively affects terminally differentiated effector subsets. The results also show increased proportions of CD8⁺ T cells with central memory phenotype and the de-repression of stem cell-related genes across different *Suv39h1*-defective CD8⁺ T cell sub-populations. In line with these observations, *Suv39h1*-defective CD8⁺ T cells show increased memory potential, including sustained survival and increased long-term re-programming capacity, as compared to *Suv39h1*-proficient CD8⁺ T cells. We conclude that Suv39h1 plays a critical role in marking chromatin to silence stem/memory gene expression during CD8⁺ effector T cell terminal differentiation. In doing so, Suv39h1/H3K9me3 would establish an epigenetic barrier on the stem/memory gene expression program, preventing the effector re-programing into memory cells. These results open new perspectives for the manipulation of epigenetic programming of T lymphocyte identity in the context of T cell-based immunotherapies.

WS.B4.01.03

TLR9 signaling mediates functional responses induced by direct ligation of ODN 2216 in CD4+CD25- effector T cells

R. K. Sharma[1], J. Sharma[1], P. Jain[2], A. Gupta[1], N. Sachdeva[1];
[1]Post Graduate Insiitute of Medical Education and Research, Chandigarh, Chandigarh, India, [2]Drexel University College of medicine, Philadelphia, Philadelphia, United States.

Oligodeoxynucleotides (ODNs) are established TLR9 ligands; however, their functional responses in CD4+ T cells are believed to be independent of TLR9 and MyD88. We have recently demonstrated activation of TLR9 signaling after ODN 2216 ligation in CD4+ effector T (Teff) cells. However, the consequences of direct ODN ligation on the immunophenotype of CD4+ Teff cells remain to be elucidated. In view of this, we looked upon the functional responses in CD4+ Teff cells after stimulation with ODN 2216 and the role of TLR9 signaling in the observed changes in cellular phenotype. Firstly, TLR9 expression in CD4+ Teff cells was regulated by downstream molecules of TLR9 signaling in a feedback controlled fashion. Next, we observed a TLR9 signaling dependent increase in proliferation of CD4+ Teff cells stimulated using ODN 2216.
Also, we observed increased synthesis of immunoregulatory molecules including TGF-β, CTLA4 and IL-10, resulting in an anti-inflammatory phenotype similar to the Th3 type of regulatory T cells. These Th3 like cells were able to suppress the proliferation of untreated Teff cells. The expression of predominant immunoregulatory cytokine, TGF-β was found to be dependent on molecules involved in TLR9 signaling in these cells, with exception of MyD88. Collectively, our results demonstrate that ODN 2216 induced functional responses in CD4+ Teff cells depend upon TLR9 signaling pathway resulting in modulation of Teff cells. Our findings thus, pave the way for future research to explore direct modulation of adaptive immune cells, using innate immune ligands, to subvert exaggerated inflammatory/autoimmune responses.

WS.B4.01.04

The T cell genome in 3D: how higher order chromatin structures regulate virus-specific T cell differentiation

B. E. Russ[1], *M. Olshansky*[1], *Z. He*[2], *J. Paulsen*[3], *S. Tomei*[1], *J. Li*[1], *P. Collas*[3], *C. Murre*[1], ***S. J. Turner***[1];
[1]Biomedical Discovery Institute, Clayton, Australia, [2]University of California, San Diego, San Diego, United States, [3]University of Oslo, Oslo, Norway.

Infection triggers large-scale changes in the phenotype and function of virus-specific CD8+ T cells ensuring that they acquire the necessary lineage specific functions critical for immune clearance of the pathogen. Whilst the molecular mechanisms that control these changes are becoming apparent, how they combine and contribute to regulate CD8+ T cell differentiation is still unclear. Genome wide mapping of chromatin interactions (HiC), histone PTMs (ChIP-seq) and chromatin accessibility (ATAC-seq) within immature thymocytes, naive effector and memory virus-specific CD8+ T cells demonstrated that maturation of higher order chromatin structures occurs upon differentiation of CD8+ T cells from an immature to mature state. Interestingly, the chromatin structure within naïve CD8+ T cells appear to be pre-configured in a lineage-specific way, both at the level of histone PTMs and higher order chromatin contacts. This genomic pre-configuration is then associated with targeted epigenetic maturation of lineage-specific genomic elements upon T cell activation, thus implying that the outcome of CD8+ T cell differentiation is largely pre-determined. These data have implications better understanding of the molecular events, and their regulation, that occur during the generation of effective T cell responses and establishment of immunological memory.

WS.B4.01.05

Deep phenotyping of colorectal cancers by high-dimensional mass cytometry reveals tumor-specific immune landscapes

N. L. de Vries[1,2], *V. van Unen*[1], *T. R. Abdelaal*[3,1], *M. E. van Herk*[1], *R. van der Breggen*[1], *A. Farina Sarasqueta*[1], *K. C. Peeters*[1], *T. Höllt*[3,1], *B. P. Lelieveldt*[3,1], *F. Koning*[1], *N. F. de Miranda*[1];
[1]Leiden University Medical Center, Leiden, Netherlands, [2]TECObiosciences GmbH, Landshut, Germany, [3]Delft University of Technology, Delft, Netherlands.

Introduction: Immune checkpoint blockade has revolutionized cancer treatment. However, clinical outcomes are highly variable as only a proportion of cancer patients benefit. As such, an in-depth understanding of the immune cell populations that participate in the process of tumorigenesis is necessary. The aim of this study is to unravel local and systemic immune profiles of colorectal cancer (CRC) using high-dimensional immunophenotyping by mass cytometry.
Materials and Methods: The expression of 36 immune cell markers was simultaneously assessed at the single-cell level by mass cytometry in tumor tissues, tumor-associated lymph nodes, adjacent normal mucosa, and peripheral blood samples from 18 CRC patients. Cytosplore and HSNE (Hierarchical Stochastic Neighbor Embedding) analyses were carried out to identify and visualize the immune composition in the tissues.
Results: We identified 218 phenotypically distinct immune subsets, including tumor-resident CD103⁺PD-1⁺ cytotoxic, helper, and gammadelta T cells, CD4⁺ICOS⁺CD27⁻ T cells, and the recently described lineage-negative CD7⁺CD127⁻CD45RO⁺CD56⁺ intermediate-innate lymphoid cells (int-ILCs), all with an activated phenotype. These cells were exclusively found in tumor tissues. Unsupervised clustering of the tissues based on the composite immune profile separated mismatch-repair (MMR)-deficient from MMR-proficient CRCs, and showed strong correlations between the presence of int-ILCs and the CD103⁺PD-1⁺ and ICOS⁺ T cell populations in MMR-deficient CRCs.
Conclusions: High-dimensional immunophenotyping of CRCs reveals tumor-specific immune signatures and points towards a coordinated adaptive and innate immune response to CRC. Previously unappreciated immune cell populations further differentiate the two main pathways of CRC tumorigenesis, and suggests a multi-targeted exploitation of their anti-tumor properties in a therapeutic setting.

WS.B4.01.06

ATTACK, a novel bispecific T cell-recruiting antibody with trivalent EGFR binding and monovalent CD3 binding for cancer immunotherapy

S. Harwood[1], ***S. Lykkemark***[1], *A. Ana Alvarez-Cienfuegos*[2], *N. Natalia Nuñez-Prado*[1], *M. Compte*[3], *S. Hernandez-Perez*[4], *N. Merino*[5], *J. Bonet*[6], *R. Navarro*[7], *P. M. van Bergen en Henegouwen*[8], *K. Mikkelsen*[1], *K. Mølgaard*[1], *F. Jabs*[1], *L. Sanz*[7], *F. J. Blanco*[5], *P. Roda-Navarro*[4], *L. Alvarez-Vallina*[1];
[1]Aarhus University, Aarhus, Denmark, [2]fMolecular Immunology Unit, Hospital Universitario Puerta de Hierro, Majadahonda, Spain, [3]Leadartis, Madrid, Spain, [4]Universidad Complutense de Madrid, Madrid, Spain, [5]CIC bioGUNE, Derio, Spain, [6]Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland, [7]Hospital Universitario Puerta de Hierro, Majadahonda, Spain, [8]Utrecht University, Utrecht, Netherlands.

The redirection of T cell activity using bispecific antibodies is one of the most promising cancer i mmunotherapy approaches currently in development, but it is limited by cytokine storm-related toxicities, as well as the pharmacokinetics and tumor-penetrating capabilities of current bispecific antibody formats. Here, we have engineered the ATTACK (Asymmetric Tandem Trimerbody for T cell activation and Cancer Killing), a novel T cell-recruiting bispecific antibody which combines three EGFRbinding single-domain antibodies (VHH; clone EgA1) with a single CD3-binding single-chain variable fragment (scFv; clone OKT3) in an intermediate molecular weight package. The two specificities are oriented in opposite directions in order to simultaneously engage cancer cells and T cell effectors, and
thereby promote immunological synapse formation. EgA1 ATTACK was expressed as a homogenous, nonaggregating, soluble protein by mammalian cells and demonstrated an enhanced binding to EGFR, but
not CD3, when compared to the previously characterized tandem bispecific antibody which has one EgA1VHH and one OKT3 scFv per molecule. EgA1 ATTACK induced synapse formation and early signaling
pathways downstream of TCR engagement at lower concentrations than the tandem VHH-scFv bispecific antibody. Furthermore, it demonstrated extremely potent, dose-dependent cytotoxicity when retargeting human T cells towards EGFR-expressing cells, with an efficacy over 15-fold higher than that of the tandem VHH-scFv bispecific antibody. These results suggest that the ATTACK is an ideal format for the development of the next-generation of T cell-redirecting bispecific antibodies.

WS.B4.02 Targeting checkpoints

WS.B4.02.01
Improving anti-CD137 immunotherapy against multiple myeloma through PD1 blockade

***A. C. Pichler**;*
Centre de Recherches en Cancérologie de Toulouse, Toulouse, France.

Multiple Myeloma (MM) is the second most common hematological malignancy in the world. MM is characterized by the development of malignant plasma cells within the bone marrow (BM). Despite new therapies, there is no cure and therefore a critical need to find new treatments. Agonist monoclonal antibodies (mAb) targeting the activation-induced costimulatory molecule CD137 (4-1BB) expressed by CD8+ T lymphocytes were recently shown to represent a promising agent against myeloma[1]. In this study, we dissected the immunological mechanisms implicated in anti-CD137 therapy in order to potentiate the anti-MM activity of this agent. Using the most relevant mouse model for MM, the Vk*MYC model, we found that anti-CD137 therapy induced CD8+ T cell activation, effector T cell expansion and anti-MM activity. However, we found that anti-CD137 mAbs was progressively accompanied with T cell dysfunctions and MM relapse. We found that anti-CD137 mAbs strongly increased the expression of immune checkpoints such as PD-1, Lag3 and Tigit that may directly account for T cell defects and myeloma outgrowth. The loss of proliferative capacity and cytokine secretion by PD1+ CD8+ T cells isolated from anti-CD137 treated mice confirmed this hypothesis. In addition, we showed that in vivo PD-1 blockade increased effector CD8+ T cells expansion induced by anti-CD137 mAb. Furthermore anti-PD1 mAb increased significantly the survival of MM bearing mice treated with anti-CD137 mAb. Altogether our data reveal that anti-CD137 promote an exhaustion program associated with PD-1 and that PD1 blockade could potentiate anti-CD137 treatment and represent a new therapeutic strategy against MM.

WS.B4.02.02
PD-1 Signals are Critical for Maintenance of Functional CD8 T Cell Memory

***S. Sarkar**[1], Y. Yuzefpolskiy[2], F. Baumann[3], K. Ernst-Bernhard[1], M. Prlic[4], P. Nghiem[2], S. Riddell[4], M. Sedensky[1], P. Morgan[1], M. C. Jensen[1], V. Kalia[1];*
[1]Seattle Children's Research Institute, Seattle, United States, [2]University of Washington School of Medicine, Seattle, United States, [3]Pennsylavania State University, University Park, United States, [4]Fred Hutchinson Cancer Research Center, Seattle, United States.

Inhibitory signaling in dysfunctional/exhausted cytotoxic T lymphocytes through the PD-1 axis is well established in diseases with chronic antigen. While PD-1 is expressed at the highest levels during priming of both acute and chronic infections, its role in long-lived antigen-independent T cell memory remains undefined. Paradoxical to its role as an inhibitory receptor, here we show that during priming and activation, PD-1 expression has minimal impact on the proliferation, size, polyfunctionality and effector program of T cells. Instead, our studies reveal an unexpected requirement of PD-1 in the maintenance of functional T cell memory, when it is expressed at significantly lower levels than recently activated or exhausted CD8 T cells. Loss of T cell-intrinsic PD-1 signals led to a striking defect in homeostatic renewal, thus resulting in a precipitous decline and near ablation of the memory pool.
Notably, in the setting of PD-1 checkpoint blockade immunotherapy for chronic viral infection, where the exhausted CTLs regained function as expected, there was significant attrition of pre-existing functional memory cells to a previously administered vaccine. Metabolically, PD-1 signals were necessary to drive the critical switch from anabolic glycolysis to fatty acid oxidation program needed for bioenergetics of quiescent memory sustenance. These studies define PD-1 as a key metabolic regulator of protective T cell immunity, and have clinical implications for pre-existing T cell memory to prior infections and vaccinations during PD-1 checkpoint-blockade immunotherapy in cancer.

WS.B4.02.03
Combination therapy with anti-PD-L1 antibody and depletion of regulatory T cells during acute viral infections results in improved virus control but lethal immunopathology

*M. Drabczik-Pluta[1], P. David[1], E. Pastille[2], T. Knuschke[2], T. Werner[1], N. Honke[3], A. M. Westendorf[2], K. S. Lang[3], U. Dittmer[1], **G. Zelinskyy**[1];*
[1]Institute for Virology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany, [2]Institute for Microbiology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany, [3]Institute of Immunology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany.

Inhibitory checkpoints like PD-1/PD-L1 and regulatory T cells (Tregs) are important suppressive mechanisms preventing immunopathology by damping immune responses against acute viral infections. Recently, combination therapy blocking these inhibitory mechanisms was induced into the clinic for the treatment of tumor diseases. It was previously reported that an experimental combination therapy targeting PD-L1 and Tregs was also effective in chronic viral infections. However, whether such a therapy is efficient during an acute infection remains to be investigated. In the current study, depletion of Tregs combined with PD-L1 and Tim-3 blocking antibodies was used during acute Friend Retrovirus infection of mice, which normally develop only transient splenomegaly after infection. The combinatorial treatment resulted in a dramatic expansion of cytotoxic CD4+ and CD8+ T cells and a subsequent reduction of viral loads in infected organs. However, limited viral replication was accompanied by a rapid development of lethal gastrointestinal immunopathology. Similar results were obtained after combination therapy in influenza virus infected mice. Treated mice efficiently controlled influenza virus, nonetheless they died of immunopathology in the lung likely mediated by cytotoxic CD8+ and CD4+ T cells. Our findings demonstrate that multiple mechanisms control the T cell response against acute viral infections, even in those infections that normally cause only mild clinical symptoms. Immunopathology is the main complication in cancer patients treated with immune checkpoint blockers. Acute infections can strongly enhance the immunopathology associated with combination immunotherapy and therefore effective measures for infection prevention should be applied in cancer patient undergoing such treatments.

WS.B4.02.04
Plasticity of Tc17 cells is regulated by CTLA-4 via STAT1/3

***A. Arra**[1], H. Lingel[1], B. Kuropka[2], T. Fischer[3], C. Freund[2], M. Pierau[1], M. Brunner-Weinzierl[1];*
[1]Department of Pediatrics, Otto-von-Guericke-University, Magdeburg, Germany, [2]Leibniz-Institut für Molekulare Pharmakologie & Freie Universität, Berlin, Germany, [3]Department of Hematology and Oncology, Otto-von-Guericke-University, Magdeburg, Germany.

Blockade of CTLA-4 on $CD8^+$ T-cells is demonstrated to be of particular importance in enhancing effector functions of Tc1 cells by secretion of granzymeB and cytokines IFNγ and TNFα. Nevertheless, the role of CTLA-4 in regulating Tc17 cells, which are generally less cytotoxic in nature but are shown to exhibit strong anti-tumor activity due to their highly plastic nature to acquire Tc1 characteristics with increased persistence, is not completely understood.
B16-melanoma model was used to investigate the effects of CTLA-4 on Tc17 cell mediated control of tumor growth. CTLA-4-mediated phosphorylation of targets in Tc17 cells was analyzed using PepScan screen. ChIP-qPCR was performed to determine STATs competence in binding to IL-17 promoter.
Tc17 cells lacking CTLA-4 signaling displayed limited activation of STAT3, leading to compromised production of its target gene products such as IL-17, IL-23R and RORγt. Upon re-stimulation with IL-12, these cells displayed faster downregulation of Tc17 hallmarks and acquire Tc1 characteristics, which are known to correlate with tumor control.
Mechanistically, in primary and re-stimulated Tc17 cells, STAT3 binding to IL-17 promoter was strongly augmented by CTLA-4, associated with less binding of STAT5 and reduced relative activation of STAT1, which is known to block STAT3 activity. Consistent with these findings, inhibition of CTLA-4-induced STAT3 activity reversed enhancement of signature Tc17 gene products, rendering Tc17 cells susceptible to conversion to Tc1-like cells with enhanced cytotoxic potential.
CTLA-4 critically shapes the characteristics of Tc17 cells by regulating relative amounts of pSTAT1/3, which provides new perspectives to enhance cytotoxicity of antitumor responses.

WS.B4.02.05
De novo DNA methylation programming restrains T cell rejuvenation during immune checkpoint blockade therapy

*H. Ghoneim, A. Moustaki, H. Abdelsamed, Y. Fan, P. Thomas, E. Stewart, S. Federico, **B. A. Youngblood**;*
St Jude Children's Research Hospital, Memphis, United States.

Immune-checkpoint blockade (ICB)-mediated rejuvenation of CD8 T cell effector functions has emerged as one of the most promising frontiers for treating cancer and chronic infections. However, antigen-specific T cells that have experienced prolonged antigen exposure are often terminally differentiated, and have a limited capacity to mount an effector response during ICB treatment. Such exhaustion of effector potential is a major impediment of current T cell based immunotherapy efforts. Using in vivo mouse models of tumor and chronic viral infection, we assessed the role of *de novo* epigenetic programming in establishing ICB-refractory exhausted T cells. We observed that genetic deletion of the de novo DNA methyltransferase, Dnmt3a, in T cells at the effector stage of an immune response to chronic lymphocytic choriomeningitis virus (LCMV) infection allowed antigen-specific T cells to remain highly functional despite expressing high levels of PD-1 and having prolonged exposure to antigen. Quite strikingly, PD-1 blockade treatment of chronically infected animals resulted in massive expansion of PD-1+ Dnmt3a-deficient antigen-specific T cells. Whole-genome methylation profiling of WT and Dnmt3a-deficient LCMV-specific CD8 T cells identified *de novo* DNA methylation programs that are coupled to development of ICB-nonresponsive virus and tumor-specific T cells. Building upon these findings, we have identified de novo epigenetic programs acquired in human tumor-associated PD-1hi CD8 T cells. Collectively, these data establish Dnmt3a-mediated de novo DNA methylation programming as a key regulator in establishing ICB-refractory exhausted CD8 T cells and highlights epigenetic reprogramming of T cells as a novel approach to enhance T cell-based cancer therapies.

WS.B4.02.06

Mitochondrial morphological and functional reprogramming following CD137 (4-1BB) co-stimulation

I. Etxeberria[1], *S. Labiano*[1], *S. Garasa*[1], *E. Santamaría*[1,2], *A. Rouzaut*[1], *M. Enamorado*[3], *A. Azpilikueta*[1,4], *S. Inoges*[1], *E. Bolaños*[1,4], *M. Aznar*[1], *A. Sánchez-Paulete*[1,4], *D. Sancho*[3], *I. Melero*[1,4], *A. Teijeira*[1,4];
[1]*Center for Applied Medical Research (CIMA), Pamplona, Spain,* [2]*CIBEREHD. Centro Virtual de la Investigación Biomédica en red de enfermedades hepáticas y digestivas, Madrid, Spain,* [3]*Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain,* [4]*CIBERONC. Centro Virtual de la Investigación Biomédica en red de Oncología., Madrid, Spain.*

CD137 (4-1BB) is a costimulatory receptor of the TNFR family expressed by T and NK lymphocytes whose function is exploitable for cancer immunotherapy. Mitochondria regulate function and survival of T lymphocytes. Herein, we show that CD137 co-stimulation provided by agonist mAb and CD137L (4-1BBL) induces mitochondria enlargement that results in enhanced mitochondrial mass and transmembrane potential in human and mouse CD8+ T cells. Such mitochondrial changes increase T-cell respiratory capacities and are critically dependent on mitochondrial fusion protein OPA-1 expression. Mass and function of mitochondria in tumor-reactive CD8 T cells from cancer-bearing mice is invigorated by agonist anti-CD137 mAb. In fact, mitochondrial mass and function are baseline depressed in CD137-deficient tumor reactive T-cells. Furthermore, tumor rejection induced by the synergistic combination of adoptive T-cell therapy and anti-CD137 antibodies is critically dependent on OPA-1 expression in transferred CD8 T cells. Moreover, stimulation of CD137 with anti-CD137 mAb in short-term cultures of human tumor-infiltrating lymphocytes leads to mitochondria enlargement and increased transmembrane potential. Collectively these data point at a critical link between mitochondrial morphology, function and enhanced anti-tumor T-cell effector activity upon CD137 co-stimulation.

WS.C1.01 Regulation in tissue specific autoimmunity 1

WS.C1.01.01

Human skin-resident and functionally competent memory T cells survive myeloablation preceding allogeneic hematopoietic stem cell transplantation

J. Strobl[1], *N. Bayer*[1], *M. Mayrdorfer*[1], *B. Reininger*[1], *L. Hammerl*[1], *S. Saluzzo*[1], *P. Kalhs*[2], *W. Rabitsch*[2], *G. Hopfinger*[2], *G. Stary*[1];
[1]*Department of Dermatology, Medical University of Vienna, Vienna, Austria,* [2]*Department of Internal Medicine I, Bone Marrow Transplantation Unit, Medical University of Vienna, Vienna, Austria.*

Myeloablative conditioning and subsequent allogeneic hematopoietic stem cell transplantation (HSCT) present unique conditions in the human system to study longevity, residency and repopulation capacities of tissue T cells. Therefore, we profiled immune cell dynamics in 45 patients receiving HSCT as treatment for hematological malignancies.
Skin biopsies and peripheral blood were taken at 5 pre-defined time points (before start of treatment, day of transplantation, 2/14/52 weeks after HSCT) and analyzed using flow cytometry, tissue immunofluorescence and low-input RNA-sequencing of purified cell subsets. Additionally, skin sections of patients receiving sex-mismatched donor cells were assessed for X/Y-chimerism by fluorescence-in-situ-hybridization up to 8 years post transplantation.
Upon myeloablative treatment, recirculating immune cells were eliminated in skin and peripheral blood and resurfaced 2-14 weeks after transplantation of donor cells. Strikingly, epidermal and dermal αβ T cells expressing residency markers remained stable throughout all time points analyzed. This skin-resident subset was largely $CD4^+$, displayed proliferative potential after TCR stimulation and was competent of cytokine production. Furthermore, skin-resident T cells of the recipient constituted 35% of T cells at time of full immunological recovery (14 weeks post-transplant) and coexisted with donor T cells up to 8 years after engraftment.
Our results combine data of a unique clinical setting with in-depth cell profiling using RNA-sequencing and imaging techniques, painting a detailed picture of skin-resident cells as a model for T cell turnover in peripheral organs. Thus, we were able to identify a remarkably resistant and long-lived T cell population with implications for numerous inflammatory conditions including graft-versus-host-reaction.

WS.C1.01.02

Phenotypic, molecular and functional characterisation of pro-inflammatory IL-17+ CD8+ T (Tc17) cells in psoriatic arthritis

K. J. Steel[1], *U. Srenathan*[1], *L. E. Durham*[1], *S. Wu*[1], *M. L. Ridley*[1], *E. Chan*[2], *B. W. Kirkham*[2], *L. S. Taams*[1];
[1]*Centre for Inflammation Biology and Cancer Immunology (CIBCI), Dept Inflammation Biology, School of Immunology & Microbial Sciences, London, United Kingdom,* [2]*Department of Rheumatology, Guy's & St Thomas' Hospital, London, United Kingdom.*

Introduction: Psoriatic arthritis (PsA) is an inflammatory joint/skin disease. Genetic associations implying a role for CD8+ T-cells (*HLA-B, RUNX3)* and the IL-23/IL-17 axis (*IL12B, IL23R, TRAF3IP2)* together with the clinical efficacy of IL-17A blockade provide a strong rationale to investigate IL-17A+CD8+ (Tc17) T-cells in patients with PsA.
Methods: Mononuclear cells were isolated from peripheral blood (PB) and synovial fluid (SF) from patients with PsA. Cells were stimulated *ex vivo* before phenotypic, transcriptional and functional analysis.
Results: Tc17 frequencies were increased in the SF vs. paired PB. Phenotypically, SF Tc17 cells (predominantly TCRαβ+) expressed skin and gut tissue-homing (CLA/CD49a/β7 integrin) and Type-17 cell associated markers (CCR6/CD161). TCRβ-sequencing of SF Tc17 cells suggested a polyclonal TCR repertoire, whilst RNA-sequencing revealed a distinct synovial Tc17 transcriptomic signature compared to PB Tc17, SF Th17 or Tc1 cells. Interestingly, Tc17 cells expressed hallmarks of tissue-resident memory T-cells (T_{RM}; CD45RA-CCR7-CD103+) whilst sorted CD8+CD69+CD103+ T_{RM} SF cells were enriched for IL-17A. Functionally, SF Tc17 cells co-expressed cytolytic molecules granzyme A and B, pro-inflammatory cytokines IFN-γ, GM-CSF, TNF-α, some IL-21 and IL-22, but little anti-inflammatory IL-10.
Conclusion: We describe a novel phenotypic and molecular signature for PsA synovial Tc17 cells. We also demonstrate, to our best knowledge for the first time, the presence of IL-17A-producing T_{RM} cells in the PsA joint. Functionally, Tc17 cells exhibit cytolytic potential and express pro-inflammatory cytokines, suggesting these cells are important contributors to the pathogenesis of PsA.
Research supported by the KHP R&D Challenge Fund, MRC, NIHR BRC, Arthritis Research UK and Novartis.

WS.C1.01.03

Lymphoid neogenesis in kidneys during lupus: involvement of CXCR3-expressing T cells

R. VEBER[1], *C. LE COZ*[1], *S. LECOMTE*[1], *F. MONNEAUX*[1], *K. A. FENTON*[2], *H. DUMORTIER*[1];
[1]*Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France,* [2]*Institute of Medical Biology, Tromso, Norway.*

Introduction: Tertiary Lymphoid Organs (TLOs) can be observed in pathological situations such as cancer, infections, graft rejection or autoimmune diseases but the mechanisms leading to their formation remain poorly understood. Our laboratory works on lupus, a chronic and systemic autoimmune disease leading to multiple organ failures among which severe kidney injuries. We have evidenced the presence of functional TLOs in the kidneys of the NZB/W spontaneous lupus mouse model (unpublished). The aim of the present study was to determine the early molecular and cellular mechanisms underlying renal TLO development in lupus.
Materials and methods: Young to old diseased NZB/W and age-matched healthy control BALB/c mice were compared and the leukocyte infiltrates present in their kidneys were characterized by flow cytometry, confocal microscopy and qPCR analyses.
Results: We demonstrate that TLO development takes place very early during the disease. Small kidney leukocyte infiltrates can be visualized in the absence of glomerular deposits and before the apparition of detectable circulating autoantibody levels. Among the first infiltrating cells, we found a majority of activated-memory T cells expressing the inflammatory chemokine receptor CXCR3. These T cells are likely attracted by the three CXCR3 ligands as we describe that CXCL9, 10 and 11 are produced in the glomeruli of very young NZB/W.
Conclusions: Altogether, our data suggest that TLO neogenesis takes place at a very early stage of disease development and that blocking T cell infiltration in the kidneys could impair TLO development and help preventing kidney dysfunction in lupus.

WS.C1.01.04

Intracapillary immune complexes recruit and activate slan-expressing $CD16^+$ monocytes in human lupus nephritis

F. Olaru[1], *T. Döbel*[1], *A. Lonsdorf*[1], *A. Enk*[1], *H. Gröne*[2], ***K. Schäkel***[1];
[1]*Department of Dermatology, Heidelberg, Germany,* [2]*DKFZ, Heidelberg, Germany.*

Lupus nephritis is a major cause of morbidity in patients with systemic lupus erythematosus. Among the different types of lupus nephritis intracapillary immune complex (IC) deposition and accumulation of monocytes are hallmarks of lupus nephritis class III and IV. The relevance of intracapillary ICs in terms of monocyte recruitment and activation, as well as the nature and function of these monocytes are not well understood. Monocytes are now classified based on their gradual differences in CD14 versus CD16 expression: classical $CD14^{++}CD16^-$, intermediate $CD14^+CD16^+$- and non-classical $CD14^-CD16^{++}$-monocytes. Within the population of $CD16^+$ LIN^- $HLA\text{-}DR^+$ leukocytes (non-classical $CD14^-CD16^{++}$-monocytes) our group defined the population of 6-sulfo LacNAc (slanMo) expressing cells. For the early focal form of lupus nephritis (class III) we demonstrate a selective accumulation of the proinflammatory population of 6-sulfo $LacNAc^+$ (slan) monocytes (slanMo), which locally expressed TNF-α. In flow chamber experiments, as well as in an in vivo model of IC-induced glomerulonephritis immobilized ICs induced a direct recruitment of slanMo from the microcirculation via interaction with Fc gamma receptor IIIA (CD16). Interestingly, intravenous immunoglobulins blocked CD16 and prevented cell recruitment. Engagement of immobilized IC by slanMo induced the production of neutrophil attracting chemokine CXCL2 as well as TNF-α which, in a forward feedback loop stimulated endothelial cells to produce the slanMo recruiting chemokine CX3CL1 (fractalkine). In conclusion, we observed that expression of CD16 equips slanMo with a unique capacity to orchestrate early IC-induced inflammatory responses in glomeruli and identified slanMo as a pathogenic proinflammatory cell type in lupus Nephritis.

WS.C1.01.05

Tbet provides advantage to Tregs for homing into type 1 inflammation sites in vivo

***B. AKKAYA**, M. Akkaya, A. H. Holstein, J. Al Souz, M. P. Maz, E. M. Shevach;*
NIH, Bethesda, United States.

T regulatory cells (Tregs) comprise subgroups that respond to microenvironmental cues. One of such is the Tbet$^+$ Tregs, which have adapted the master transcriptional factor of T helper 1 (Th1) cells. Although Tbet is considered as a prerequisite for restraining Th1 responses, there are still gaps in our understanding for how. To address this, we imaged Tbet$^+$ Tregs over the course of a Th1 response in live lymph node sections of a Tbet.ZsGreen-Foxp3.RFP reporter mouse during Type 1 inflammation. We showed that unlike Tbet$^-$ Tregs, majority of which resided within the T cell zone, Tbet$^+$ Tregs localized specifically to the Interfollicular zone. We then generated Tbet$^+$ iTregs from naïve OTII- Tbet.ZsGreen-Foxp3.RFP mice, adoptively transferred into C57BL/6 recipients that had been immunized in the footpad with OVA-CFA. We found higher number of OTII-ZsGreen$^+$RFP$^+$ iTregs than neutral OTII iTregs at popliteal lymph node following OVA-CFA injection, suggesting a homing advantage provided by Tbet. Furthermore, we generated bone marrow chimeras of WT-Tbet$^{-/-}$ mice and found that Treg compartment was reconstituted equally. However, we detected fewer Tbet$^{-/-}$ Tregs in the spleens of P. Chabaudi infected chimeric mice without any defect in CD44 expression and proliferation level indicating that the defect is not due to activation status but homing. Additionally, Tbet$^+$, Tbet$^-$ and Tbet$^{-/-}$ iTregs suppressed ex vivo differentiated Th1 proliferation equally, indicating that Tbet is not required for active suppression. Taken together, we propose that Tbet provides homing advantage to Tregs in vivo without potentiating their suppressive ability once the inflammation site is reached.

WS.C1.01.06

Nox2 deficiency in CD4$^+$CD25$^+$FoxP3$^+$ T cells limits angiotensin II-induced hypertension and cardiovascular remodelling

***S. Cellone Trevelin**, A. Emerson, H. Mongue-Din, P. D. Becker, C. Ortiz, L. A. Smyth, G. Sawyer, A. Ivetic, Q. Peng, R. Elgueta, R. I. Lechler, A. M. Shah, G. Lombardi;*
King's College London, London, United Kingdom.

Introduction: Nox2 is the catalytic subunit of a multi-protein complex that generates superoxide and is known to contribute to hypertension and cardiovascular remodelling induced by angiotensin II (ANGII). Recent studies showed that Nox2 has cell-specific roles in cardiomyocytes and endothelial cells but its role in T-cell subsets is poorly understood.
Methods: We generated a novel mouse line with CD4-targeted Nox2 deficiency (Nox2$^{fl/fl}$CD4Cre$^+$) and studied the response to infusion of ANGII (1.1mg/kg/day, 14 days).
Results: As compared to littermate controls (Nox2$^{fl/fl}$), Nox2$^{fl/fl}$CD4Cre$^+$ mice showed an increased proportion of CD4$^+$CD25$^+$FoxP3$^+$ Tregs in the heart and aorta at baseline and after ANGII infusion (multicolour flow cytometry). This was accompanied by a reduction in infiltrating CD4$^+$ROrγT$^+$ and CD8$^+$FoxP3$^-$ Teffs, and inhibition of AngII-induced hypertension, heart fibrosis and cardiomyocyte hypertrophy. The protection in Nox2$^{fl/fl}$CD4Cre$^+$ mice was reversed by depleting Tregs with an anti-CD25/PC61 antibody. *In vitro* studies revealed that Nox2-deficient CD4$^+$CD25$^+$Tregs suppress proliferation of CD4$^+$CD25$^-$Teffs more than WT-Tregs, and inhibits the IL-17 production stimulated by co-culture of Teffs with antigen presenting cells in the presence of anti-CD3. Nox2-deficient Tregs also had higher nuclear levels of FoxP3 and p65/NF-κB, and increased mRNA levels of CTLA-4, CD39, CD73, GITR and CD25, than WT-Tregs. In adoptive transfer experiments, Nox2-deficient Tregs were more potent at inhibiting ANGII-induced hypertension and heart fibrosis than WT-Tregs.
Conclusion: Nox2 deficiency in Tregs limits cardiovascular remodelling induced by ANGII by suppressing infilitration of Teffs. These results suggest that targeting Nox2 in Tregs might be a useful approach in cardiovascular disorders.

WS.C1.02 Immune regulation at mucosal sites

WS.C1.02.01

Intestinal Secretory Leukocyte Protease Inhibitor expression is increased in pediatric inflammatory bowel disease patients and is unfavorable during murine colitis

***S. Nugteren**, C. L. Menckeberg, Y. Simons-Oosterhuis, D. J. Lindenbergh-Kortleve, L. A. van Berkel, H. C. Raatgeep, L. M. Costes, L. de Ridder, J. C. Escher, J. N. Samsom;*
Erasmus Medical Center, Rotterdam, Netherlands.

Secretory Leukocyte Protease Inhibitor (SLPI) is an NF-κB inhibitor produced by epithelial cells in response to microbial signals. Previously, we have shown that knockdown of SLPI in intestinal epithelial cells elicits NF-κB activation and subsequent chemokine production, indicating that SLPI inhibits epithelial activation. Crucially, upon increased bacterial pressure at the epithelial border, as seen in mice deficient for one of the major colonic mucins, we detected increased intestinal SLPI expression. As inflammatory bowel disease (IBD) is driven by aberrant host microbial interactions, we hypothesized that intestinal SLPI expression is increased in IBD patients. Furthermore, we questioned whether intestinal SLPI is beneficial or deleterious during intestinal inflammation.
In therapy-naïve IBD patients, we observed increased SLPI mRNA and protein expression in macroscopically inflamed intestinal tissue, compared to macroscopically non-inflamed and non-IBD tissue. To investigate the role of SLPI during intestinal inflammation, murine colitis was induced using dextran sodium sulphate (DSS) in wild type (WT) and SLPI knockout (KO) mice. In WT mice we observed increased fecal SLPI excretion and increased intestinal *Slpi* transcripts during the peak of inflammation. Crucially, SLPI KO mice were less affected by DSS colitis compared to WT littermates, as evidenced by reduced weight loss and lower colonic expression of the pro-inflammatory cytokines TNFα and IL-1β. Altogether, our findings suggest that increased intestinal SLPI expression during intestinal inflammation is disadvantageous to the host. In IBD patients, high intestinal SLPI expression and consecutive reduced NF-κB-mediated epithelial activation may inhibit anti-microbial host defense leading to more severe disease.

WS.C1.02.02

ATF3 is crucial for intestinal mucosal immunity during homeostasis and stress

***D. Glal**;*
Institute of Biomedical sciences, Taipei, Taiwan.

Activating Transcription Factor 3 (ATF3) is induced by a wide-range of cellular stresses, and found to be involved in many critical human diseases including cancer, atherosclerosis, infections, cardiac hypertrophy, and hypospadias. Interestingly, a recent microarray study has also identified ATF3 up regulation in patients with active inflammatory bowel disease (IBD). Using mouse model and cell system, we have found that ATF3 is central to intestinal homeostasis at steady state and protection during inflammation. This was shown, in naïve ATF3-deficient mice, by decreased crypts numbers and colon length indicating defective cellular stemness and regeneration to maintain a healthy tissue mass. Using DSS-induced colitis, ATF3-deficient mice showed lethal disease activity characterized by a dramatic loss of epithelial architecture and crypt structure, poorness of proliferation/repair machine, and strenuous apoptosis, which has been effectively ameliorated by rectal transplantation of wild-type organoids indicating that the intestinal protective role of ATF3 is mainly occur through maintaining epithelial cell integrity. Paneth cells and stem cells form a niche to orchestrate the differentiation, proliferation, and repair in the intestine. Using electron microscopy, we have identified loss/degeneration of Paneth cell granules, which are the main source of antimicrobial peptides (AMPs), indicating malfunctioning of intestinal Paneth/Stem cell network. Impaired cell proliferation rate and wound healing, and loss of AMPs production have been found also to be consistent in ATF3-/- epithelial cell line. Collectively, our findings provide a new insight for ATF3 in terms of intestinal mucosal immunity; a favorable role that brings ATF3 to be a potential competent for further IBD-related researches.

WS.C1.02.03

The transcription factor MAF regulates homeostasis in colonic T cells

*C. Imbratta[1], M. Leblond[1], D. Velin[2], D. E. Speiser[1], **G. VERDEIL**[1];*
[1]University of Lausanne, Epalinges, Switzerland, [2]Lausanne University Hospital, Lausanne, Switzerland.

Maf encodes for a transcription factor belonging to the AP-1 family. In CD4 T helper cells (Th), it has a role in *il-4* transcriptional regulation in Th2, in Th17 cells through the regulation of *il-10* and *il-23r* expression and in Tfh cells together with the transcription factor *bcl6*. We recently demonstrated that *maf* is induced in CD4 and CD8 T cells in melanoma, leading to a "dysfunctional" state of the cells. The role of *maf* in regulatory T cells (Treg) is less clear. A subset of Treg cells expressing both Foxp3 and RORγt has been described. Present in the gut, ROR-γt+ Treg cells have an enhanced suppressive activity compared to ROR-γt- Treg cells, especially in an *in vivo* context of gut inflammation. Transcriptomic analysis of this population showed an enriched expression of *maf* in this specific Treg subset. To precise the function of *maf* in T cells *in vivo*, we studied a T-cell specific KO of *maf*. These mice developed late onset colitis correlating with the loss of ROR-γt+ Treg cells. While IL-10 expression was reduced, TNF-α and IL-17A production was increased in the colon. We found both *in vitro* and *in vivo* that Maf KO Treg cells produced less IL-10 and had impaired suppressive capacity compared to those derived from WT animals. Our data shows that MAF expression in T cells, especially ROR-γt+ Treg cells, is essential for their differentiation and to maintain their suppressive activity and thereby prevent inflammatory bowel disease by inhibiting Th1 and Th17 polarization.

WS.C1.02.04

Proinflammatory, high avidity CD4⁺ T cells with a memory-like phenotype in the human fetal intestine

N. Li[1], *V. van Unen*[1], *T. Abdelaal*[1,2], *K. Ladell*[3], *J. E. McLaren*[3], *D. A. Price*[3], *S. M. Chuva de Sousa Lopes*[1], *B. P. Lelieveldt*[1,2], *F. Koning*[1];
[1]*Leiden University Medical Center, Leiden, Netherlands,* [2]*Delft University of Technology, Delft, Netherlands,* [3]*Cardiff University School of Medicine, Cardiff, United Kingdom.*

The human fetus is thought to be protected from contact with environmental antigens prior to birth, yet evidence has emerged that CD45RO⁺ T cells are present in the human fetal intestine. We applied a combination of mass cytometric analysis, single-cell sequencing, and functional studies to gain comprehensive insight into the heterogeneity and functionality of the CD4⁺ T cell compartment in the human fetal intestine. Using mass cytometry, we distinguished 22 distinct CD4⁺ T cell clusters, including naïve-like, regulatory, memory-like, and CD45RO⁺CD117⁺ subsets. Single-cell RNA sequencing confirmed the presence of these distinct CD4⁺ T cell subsets and further characterized their expression profiles. Strikingly, the majority of CD4⁺ T cells were CD45RO⁺ memory-like cells, expressing high levels of CD69, CD226, CXCR3, CCR4, Ki67 and CD5. The latter two markers are indicative of cell proliferation and high T cell receptor avidity, respectively. Pathway analysis revealed a strong enrichment for transcripts associated with T cell activation and regulation of the inflammatory response to antigens. In addition, these memory-like T cells showed signs of clonal expansion and were readily stimulated to produce high levels of TNF-α and some IFN-γ. Our data therefore provide evidence for the existence of a large pool of memory-like, high avidity CD4⁺ T cells with a proinflammatory phenotype in the human fetal intestine, indicative of antigenic stimulation *in utero*.
Grants and Fellowships: ZonMW grant 91112008, China Scholarship Council, and Wellcome Trust grant 100326/Z/12/Z.
1

WS.C1.02.05

The transcription factor NFATc3 promotes intestinal inflammation by suppression of regulatory T cells

K. Gerlach*, V. Popp, M. Neurath, B. Weigmann;*
Medizinische Klinik 1, Erlangen, Germany.

The transcription factor NFATc3 (Nuclear factor of activated T cells) belongs to a transcription factor family of five members. NFATc3 plays an important role in the activation and function of T cells regulating cytokine expression and cell proliferation. High numbers of NFATc3⁺ cells in the lamina propria of patients suffering from inflammatory bowel disease (IBD) point out the regulatory role of this transcription factor in mucosal inflammation and led us to investigate its function in colitis. Deficiency of NFATc3 in the oxazolone-induced colitis model suppressed induction of intestinal inflammation. Staining for Caspase3 showed less proapoptotic cells in the colon of NFATc3 KO mice whereas apoptotic cells were significantly increased. Additionally, we found a higher number of FoxP3⁺ T cells in the colon of NFATc3 KO mice suggesting that NFATc3 controls regulatory T cells. As Tregs have been shown to prevent and cure intestinal inflammation caused by the adoptive transfer of naïve T cells in immunodeficient RAG1 knockout mice, we next analysed the relation between Tregs and NFATc3 in this colitis model. Mice receiving NFATc3-deficient naïve T cells had a later onset of inflammation than mice reconstituted with wildtype T cells. Moreover, the adoptive transfer of NFATc3-deficient T cells was associated with an increased number of CD3⁺ FoxP3-expressing Tregs. In summary, the transcription factor NFATc3 crucially promotes intestinal inflammation by affecting FoxP3 expression and therefore serves as a potential target for therapy in IBD.

WS.C1.02.06

The molecular composition of IgA anti-carbamylated and anti-citrullinated protein antibodies in Rheumatoid Arthritis, point to a mucosal origin

M. A. M. van Delft[1], *M. K. Verheul*[1], *N. Levarht*[1], *L. Hafkenscheid*[1], *T. Kissel*[1], *A. Bondt*[2], *T. W. Huizinga*[2], *R. E. Toes*[2], *L. A. Trouw*[2];
[1]*Miss, Leiden, Netherlands,* [2]*Mr, Leiden, Netherlands.*

The presence of autoantibodies targeting post-translationally modified proteins, such as citrullinated (ACPA) and carbamylated (anti-CarP antibodies) proteins, are a hallmark of RA. As these autoantibody responses target homologous structures (citrulline and homo-citrulline) that differ only in one CH_2-group, the IgG ACPA- and anti-CarP- responses are partially cross-reactive in some- but not in other patients. Both responses use a broad spectrum of isotypes, including IgM, IgG and IgA. To better understand the nature of these prominent autoantibodies, we investigated the molecular composition of both ACPA and anti-CarP antibodies. Moreover, the degree of cross-reactivity of these autoantibodies was investigated. Sera of anti-CarP and/or ACPA positive RA-patients were fractionated using size exclusion chromatography and tested by ELISA for anti-CarP, ACPA and total IgA and IgG.
Inhibition studies were performed to investigate the cross-reactivity between anti-CarP and ACPA. Our results show that anti-CarP antibody, rheumatoid factor and anti-*E.Coli* IgA are predominantly present as polymeric-IgA, whereas ACPA and anti-Tetanus Toxoid IgA are mostly present as monomeric-IgA. These data are intriguingly as they indicate that the anti-CarP and ACPA IgA responses are differently regulated and potentially of different origin. About 75% of the anti-CarP IgA response cannot be inhibited by citrullinated antigens and vica versa for ACPA IgA by carbamylated antigens, indicating that these autoantibodies are directed against different antigens. To conclude; our data indicate that the anti-CarP- and ACPA-autoantibody responses are differently regulated and may have a different origin, with possibly a mucosal associated origin for anti-CarP antibodies.

WS.C1.03 Cytokine and transcription factor mediated immune regulation

WS.C1.03.01

Anti-TNF treatment leads to delayed activation, maturation and proliferation of CD4+ T cells but does not confer a global suppressive phenotype

G. A. M. Povoleri[1], *S. Lalnunhlimi*[1], *K. Steel*[1], *S. Agrawal*[1], *M. Ridley*[1], *S. Kordasti*[2], *C. Roberts*[1], *L. S. Taams*[1];
[1]*Centre for Inflammation Biology and Cancer Immunology (CIBCI), Dept of Inflammation Biology, School of Immunology & Microbial Sciences, King's College London, London, United Kingdom,* [2]*Department of Haematological Medicine, King's College London, London, United Kingdom.*

We previously demonstrated that *in vitro* treatment of human CD4+ T-cells with the TNF-blocking drug adalimumab, promotes anti-inflammatory IL-10 expression. We investigated whether this effect is accompanied by changes in cellular activation, maturation, proliferation and suppressive function.
CD4+ T-cells from healthy volunteers were cultured for up to 7 days with anti-CD3/CD28 mAb stimulation, in the absence or presence of anti-TNF. Phenotypic changes were evaluated by flow cytometry and CyTOF. Gene expression changes were evaluated using existing datasets (GSE51540). For suppression assays, cells were re-isolated and added to responder T-cells, monocytes or fibroblasts.
Culturing CD4+ T-cells with anti-TNF led to decreased activation as shown by reduced frequencies of CD25+, CD69+ and HLA-DR+ cells. CD4+ T-cells also contained significantly higher CD45RA+ and lower CD45RO+ frequencies in the presence of anti-TNF. Proliferation was reduced as indicated by lower percentage of Ki67+ cells and proliferation assays. Additionally, gene expression analysis of anti-TNF-treated IL-17 or IFNγ-producing CD4+ T-cells revealed multiple pathways associated with cell proliferation and cell cycle. Kinetics experiments suggested that anti-TNF treatment leads to delayed, rather than impaired T-cell activation. Furthermore, while anti-TNF treated CD4+ T-cells did not exhibit a significant difference in suppression of T-cell proliferation and monokine production, compared to untreated cells, they displayed hyporesponsiveness, induced the IL-10 regulated molecule CD163 on monocytes and downregulated IL-8 production by synovial fibroblasts.
We demonstrate that anti-TNF treatment resulted in delayed activation, maturation and proliferation of CD4+ T-cells, but not in acquisition of a global suppressive phenotype.
Funded by Arthritis Research UK (#21139)

WS.C1.03.02

Vγ6+ γδ17 T cells home to the male reproductive tract and expand at puberty to keep pathogens at bay

H. Brigas*[1], *A. Wilharm*[*2], *I. Sandrock*[2], *T. Amado*[1], *T. Carvalho*[1], *A. Reinhardt*[2], *B. Silva-Santos*[1], *I. Prinz*[**2], *J. Ribot*[**1];
[1]*Instituto de Medicina Molecular João Lobo Antunes, Lisboa, Portugal,* [2]*Institute of Immunology, Hannover, Germany.*

γδ T cells populate multiple tissues where they make major contributions to local physiology. They have also been characterized in the female, but not in the male, reproductive tract. Here, we found that γδ T cells infiltrate the stromal tissue of the testis of naive C57/BL6 mice, expand at puberty and persist throughout life. Strikingly, this population of testicular γδ T cells selectively displayed a Vγ6+ repertoire and was highly enriched in IL-17 producers (γδ17). In fact, γδ T cells were the major source of IL-17, whereas ab T cells mostly provided IFN-γ in situ. γδ17 T cell homeostasis in the testis seemingly depended on IL-1a/IL-23 signals downstream of TLR4 expressed by resident myeloid populations. Interestingly, recent studies have shown that androgens shape the gut microbiome at puberty. Our data suggest that cues from the microbiota may drive the expansion of γδ17 T cells in the testis, as Germ-Free mice display a significant reduction in this population. Furthermore, we could induce an early γδ17 T cell expansion in the testis, before puberty, through adult male fecal transfer. We next hypothesized that testicular γδ17 T cells might contribute to tissue surveillance. We performed intra-testicular inoculation of Listeria monocytogenes, a commonly used model of orchitis. Our data indicate that infected TCRδ-/- and IL-17-/- mice display higher bacterial load and die within 4 days after infection, whereas WT controls survive. Altogether, our results identify a previously unappreciated resident testicular γδ17 T cell subset that plays a crucial role against local bacterial infection.

WS.C1.03.03

IL-27 increases the plasticity of differentiated Th17 cells by inducing IL-12R beta2 expression

A. Awasthi[1], *V. K. Kuchroo*[2];
[1]*Translational Health Science & Technology Institute, Faridabad, India,* [2]*Brigham and Womens Hospital, Harvard Medical School, Boston, United States.*

The role of Th17 cells in inducing tissue inflammation in autoimmune diseases is well established. IL-23-IL-23R-induced pathogenic T_H17 cells are critical in inducing tissue inflammation in experimental autoimmune encephalomyelitis (EAE), a mouse model of human multiple sclerosis. Interleukin (IL-) 27, an IL-12 family cytokine, has been shown to suppress tissue inflammation in EAE partly by inhibiting differentiation of T_H17 cells. In addition to inhibition the differentiation and functions of Th17 cells, IL-27 also found induce the differentiation of IL-10 producing type regulatory T cells (Tr1), which are found to play an essential role in inhibiting effector T cells function in EAE. Although the anti-inflammatory role of IL-27 was established, the precise mechanism by which IL-27 suppresses the effector functions of differentiated T_H17 cells is not well understood. Using the global gene profile data, we now show that IL-27-induced expression of IL-12Rβ2 plays a critical role in regulating the generation and functions of T_H17 cells in EAE. Moreover, IL-27-induced IL-12Rβ2 make differentiated Th17 cells responsive IL-12-mediated conversion into IFN-gamma producing T cells. IL-27 receptor deficiency leads to the inhibition of IL-12Rβ2 while enhancing the expression of IL-23R on Th17 cells, and therefore increases the IL-23 responsiveness of Th17 cells. Moreover, IL-27 is unable to inhibit T_H17 cells and development of experimental autoimmune encephalomyelitis (EAE) in *Il12rb2*$^{-/-}$ mice. Indeed, IL-27-induced expression of IL-12Rβ2 increased responsiveness of T_H17 cells to IL-12. This provides a novel mechanism by which IL-27 inhibits T_H17 cells by increasing their plasticity and responsiveness to immunosuppression.

WS.C1.03.04

TGF-β bioactivity in *Trichuris muris* homogenate induces FOXP3$^+$ T regulatory cells and inhibits Th1 and Th2 polarisation when activated

A. E. Ogunkanbi[1], *B. Eldakhakhny*[2], *J. L. Pennock*[1];
[1]*Institute of Infection, Immunity and Respiratory Medicine, Manchester, United Kingdom,* [2]*Faculty of Medicine , Department of Clinical Biochemistry, King Abdulaziz University,, Saudi Arabia, Saudi Arabia.*

The establishment of a long lasting chronic infection with helminths relies on their ability to modulate host protective immune responses by direct induction of host immunomodulatory molecules such as IL-10, Tregs cells and TGF-β. Immune modulation by helminths has been centred on induction of host regulatory responses. However, research evidence has shown that helminths themselves can encode TGF-β receptor ligands to modulate the immune response and enhance their survival. We addressed whether *T. muris* can encode TGF-β-like ligands to enhance their survival in the host using bioinformatics*, in vitro* and *in vivo* approaches. *In vitro*, acid treated worm homogenate activates TGF-β responsive cell lines. Acid activated worm homogenate also induces foxp3 expression in mouse T cells in a dose-dependent manner and reduces IL-13 and IFN-γ production during T cell polarisation. The induction of foxp3 was abolished by anti TGF-β antibody (1D11) and both the production of IL-13 and IFN-γ was restored upon addition of 1D11. Finally*, T. muris* homogenate can induce endogenous production of TGF-β both *in vivo* and *in vitro* and this is associated with the reduction in IFN-γ production. These data have shown that unique TGF-β activity is present in *T. muris* and supports the current paradigm that worms have evolved mechanisms to potentiate their survival. These data also complement and extend our current understanding of helminth immunoregulation and broaden the scope for potential therapeutics for regulation of intestinal inflammation.

WS.C1.03.05

Hobit identifies precursors of resident memory T cells within the peripheral tissues

L. Parga Vidal[1], *F. Behr*[1,2], *N. Kragten*[1], *T. Wesselink*[1], *R. Stark*[1,2], *K. van Gisbergen*[1,2];
[1]*Department of Hematopoiesis, Sanquin Research and Landsteiner Laboratory, AMC, University of Amsterdam, Amsterdam, Netherlands,* [2]*Department of Experimental Immunology, AMC, Amsterdam, Netherlands.*

Tissue-resident memory T (Trm) cells constitute a non-circulating memory subset that provides early protection against re-infection. It is unclear when, where and how these Trm develop from effector CD8 T cells. We have previously described that Hobit represents a Trm-specific transcription factor that is essential for their formation. To employ Hobit for the study of Trm differentiation, we generated a Hobit reporter mouse, which contains a “knock-in” of the fluorescent protein tdTomato and the diphtheria toxin (DT) receptor within the *Hobit* locus. We infected Hobit reporter mice with LCMV and analyzed virus-specific CD8 T cells for tdTomato expression by flow cytometry. Trm present in gut, kidney, liver and salivary glands nearly uniformly expressed tdTomato in contrast to circulating memory cells. Interestingly, tdTomato was already upregulated in a subset of effector cells located within these peripheral tissues, but not in lymph nodes, blood or spleen. To examine the potential of the tdTomato$^+$ effector cells to establish memory populations, we depleted them using DT injections at different time-points after infection. We observed a substantial and specific decrease in Trm after depletion of tdTomato$^+$ effector cells between day 7 and 10 after infection. Depletion at earlier time points did not have an impact on Trm formation. These findings show that Hobit$^+$ effector CD8$^+$ T cells are Trm precursors. Furthermore, we conclude that commitment of effector cells to the Trm lineage occurs in the peripheral tissues at the peak of the effector CD8 T cell response.

WS.C1.03.06

EOMES-positive CD4$^+$T cells are increased in *PTPN22* (1858T) risk allele carriers

K. Chemin[1], *D. Ramsköld*[1], *L. Diaz-Gallo*[1], *J. Herrath*[1], *M. Houtman*[1], *K. Tandre*[2], *L. Rönnblom*[2], *V. Malmström*[1];
[1]*Center for Molecular Medicine, Stockholm, Sweden,* [2]*Uppsala University, Uppsala, Sweden.*

The presence of the *PTPN22* risk allele (1858T) is associated with several autoimmune diseases including rheumatoid arthritis (RA). Despite a number of studies exploring the function of *PTPN22* in T cells, the exact impact of the *PTPN22* risk allele on T-cell function in humans is still unclear. In this study, using RNA sequencing, we show that, upon TCR-activation, naïve human CD4$^+$ T cells homozygous for the *PTPN22* risk allele overexpress a set of genes including *CFLAR* and *4-1BB*, which are important for cytotoxic T-cell differentiation. Moreover, the protein expression of the T-box transcription factor Eomesodermin (EOMES) was increased in T cells from healthy donors homozygous for the *PTPN22* risk allele and correlated with a decreased number of naïve CD4$^+$ T cells. There was no difference in the frequency of other CD4$^+$ T-cell subsets (Th1, Th17, Tfh, Treg). Finally, an accumulation of EOMES$^+$ CD4$^+$ T cells was observed in synovial fluid of RA patients with a more pronounced production of Perforin-1 in *PTPN22* risk allele carriers. Altogether, we propose a novel mechanism of action of *PTPN22* risk allele through the generation of cytotoxic CD4$^+$ T cells and identify EOMES$^+$ CD4$^+$ T cells as a relevant T-cell subset in RA pathogenesis.

Eur J Immunol. 2018 Apr;48(4):655-669. doi: 10.1002/eji.201747296

WS.C1.04 Regulation in tissue specific autoimmunity 2

WS.C1.04.01

Th17.1 cells preferentially recruit to the central nervous system to mediate early disease activity in multiple sclerosis

J. van Langelaar[1,2], *R. M. van der Vuurst de Vries*[1,2], *M. Janssen*[1,2], *A. F. Wierenga-Wolf*[1,2], *I. M. Spilt*[1,2], *T. A. Siepman*[1,2], *W. Dankers*[1], *G. M. Verjans*[1,3], *H. E. de Vries*[4], *E. Lubberts*[1], *R. Q. Hintzen*[1,2], ***M. M. van Luijn***[1,2];
[1]*Erasmus MC, Rotterdam, Netherlands,* [2]*MS Center ErasMS, Rotterdam, Netherlands,* [3]*University of Veterinary Medicine, Hannover, Germany,* [4]*VU University Medical Center, Amsterdam, Netherlands.*

Multiple sclerosis (MS) is mediated by pathogenic CD4+ T cells that infiltrate the CNS to promote local inflammation and demyelination. IL-17-producing CCR6+ Th cells are the main drivers of EAE, the animal model for MS. However, the functional programs of CCR6+ Th cells are heterogeneous and differ between mice and men. Here, we assessed distinct effector populations of human Th17 cells and how their recruitment to the CNS associates with MS disease onset.
Low frequencies of CCR6$^+$CXCR3$^+$ (Th1-like Th17), and not CCR6$^+$CXCR3$^-$ (Th17) effector memory cells in the blood strongly associated with rapid diagnosis of MS. In CSF, Th1-like Th17 cells were abundant and showed increased IFN-γ/GM-CSF production compared to paired CCR6$^+$ and CCR6$^-$CD8$^+$ T cells and their blood equivalents after short-term culturing. Their local enrichment was confirmed *ex vivo* using paired MS CSF and brain single-cell suspensions. Across all pro-inflammatory Th populations analyzed in the blood, a IL-17^{low}IFN-γ^{high}GM-CSF^{high} subset termed Th17.1 (CCR6$^+$CXCR3$^+$CCR4$^{-/low}$) expressed the highest levels of VLA-4, and selectively accumulated in natalizumab-treated MS patients who remained free of acute relapses. This was not found for patients who encountered relapses. The pathogenicity of Th17.1 was further supported by their predominance in early MS CSF, enhanced transmigration across human brain endothelial monolayers *in vitro*, and increased expression of IL-23R, MDR1 and granzyme B.
These findings reveal a selective contribution of Th17.1 cells to CNS inflammation and provide a strong rationale for more refined and earlier use of T cell-directed therapy in MS patients.
Funding: Dutch MS Research Foundation, Zabawas Foundation

WS.C1.04.02

4-1BB overexpression in basal keratinocytes induces the disruption of the eye´s immune privilege

E. Gonnelli[1]*, M. Kasper*[2]*, V. Kupas*[3]*, D. Bauer*[2]*, T. A. Luger*[1]*, A. Heiligenhaus*[2]*, K. Loser*[1]*;*
[1]*Department of Dermatology, Münster, Germany,* [2]*Institute of Experimental Ophtalmology at Franziskus Hospital, Münster, Germany,* [3]*MorphoSys AG, München, Germany.*

4-1BB (also called CD137 or TNFRS9) belongs to the tumor necrosis factor receptor superfamily (TNFRS), and has a crucial role as a costimulatory molecule in a variety of immune processes. The overall effect of 4-1BB/4-1BB ligand signaling is enhancing inflammatory responses. To investigate the role of 4-1BB signaling in more detail we generated a mouse model with overexpression of 4-1BB under control of the keratin-14 (K-14) promoter. Surprisingly, besides severe pruritus, K14-4-1BB tg mice spontaneously developed uveitis and anterior cataract beginning at the age of 3 weeks, which was associated with the infiltration of immune cells into the eye, finally resulting in blindness. Immunofluorescent staining as well as qPCR and FACS analysis was used to characterize the cell infiltrate, revealing that the infiltrate was mainly consisting of MHCII+F4/80+ macrophages. Moreover, by performing whole mRNA array analyses we confirmed the downregulation of genes related to tight junction formation, cell-cell interaction and transmigration that might favor the income of immune cells into the eye. Interestingly, in addition we observed the presence of markers supporting an epithelial-mesenchymal-transition (EMT) occurring in the epithelial cell layer of the anterior chamber´s lens capsule from K14-41BB tg mice strongly suggesting that the EMT might be the trigger for the infiltration of immune cells through leaky cell junctions. Together, these data indicate that 4-1BB signaling is critically involved in the development of uveitis and anterior cataract and might play an important role in disrupting the immune privilege of the eye.

WS.C1.04.03

Targeting CD146 can downregulate new blood vessel formation caused by VEGF-producing T cells in rat experimental autoimmune uveitis

G. Wildner*, M. Diedrichs-Möhring, S. Thurau;*
Section of Immunobiology, Department of Ophthalmology, University Hospital, LMU Munich, Munich, Germany.

Neovascularization in the retina as a consequence of inflammation is a major problem in the eye. We have recently shown that VEGF-producing autoreactive T cells in rat experimental autoimmune uveitis can also induce chorioretinal neovascularization (CNV). Here we investigate the expression of CD146, a molecule of endothelial tight junctions and a coreceptor of VEGFR-2, in the eye and the effect of antibody to CD146 on EAU and formation of CNV. To induce clinically monophasic uveitis with neovascularizations Lewis rats were immunized with S-Ag peptide PDSAg-CFA or adoptively transferred with PDSAg-specific T cells. After adoptive transfer anti-CD146 antibody was daily injected s.c. and uveitis intensity monitored clinically and histologically. To investigate the effect on CNV formation, anti-CD146 antibody was injected once into the anterior chamber of rats one day prior to onset of clinical disease and CNV formation determined histologically. Cryosections from rat eyes with EAU were stained with anti-CD31/PECAM and anti-CD146. In eyes with EAU CD146 was expressed in the choroid/choriocapillaris and CNV, also on some infiltrating T cells. Preventive systemic or intraocular treatment with anti-CD146 only marginally affected the intensity of intraocular inflammation, while a single intraocular injection of anti-CD146 prior disease onset significantly suppressed CNV formation. Anti-CD146 antibodies can prevent the pathological development of new vessels in the eye after a single intraocular injection in an EAU model, where autoreactive T cells produce VEGF and induce CNV. Targeting this molecule could be a new therapeutic option to prevent the growth of new blood vessels.
Support: EU grant NoE-MAIN

WS.C1.04.04

Persistence of dominant TCRâ clones in regulatory T cells derived from an autoimmune inflammatory environment

G. Mijnheer[1]*, J. Leong*[2]*, A. Boltjes*[1]*, E. Spierings*[1]*, A. Petrelli*[1]*, S. Vastert*[1]*, S. Albani*[3]*, A. Pandit*[1]*, F. van Wijk*[1]*;*
[1]*University Medical Center Utrecht, Utrecht, Netherlands,* [2]*SingHealth and Duke-NUS Graduate Medical School, Singapore, Singapore,* [3]*SingHealth and Duke-NUS Graduate Medical Schoo, Singapore, Singapore.*

Inflammation is characterized by infiltration of multiple immune cells and expansions of antigen-specific T cells. In autoimmune diseases, inflammation is often limited to specific target tissues, but within tissues, multiple sites can be affected. An important outstanding question is whether affected sites are infiltrated with the same pathogenic T cell clones and whether these clones persist over time. In Juvenile Idiopathic Arthitis (JIA) it is relatively easy to analyze cells derived from the site of inflammation, i.e. inflamed joints. Here we performed CyToF and T cell receptor (TCR) sequencing to study immune cell composition and (hyper)expansion of inflamed joint derived T cells. The samples were taken from different joints affected at the same time, and joints that were affected multiple times during the relapsing remitting course of the disease. CyTOF analyses revealed that the composition and functional characteristics of the immune infiltrates are strikingly similar between joints within one patients. Furthermore we observed a strong overlap between dominant T cell clones (Teff and even more pronounced for Treg) in inflamed joints affected at the same time, and some of the most dominant clones could also be detected in circulation. Finally, these dominant T cell clones were found to persist over time and to expand during relapses, even after full remission of the disease. These data suggest that in autoimmune disease there is auto-antigen driven expansion of both Teff and Treg clones, that are highly persistent and are (re)circulating. Therefore these dominant clones can be interesting therapeutic targets.

WS.C1.04.05

Characterization of constitutively DC-deficient mice in autoimmune responses

C. Hilpert*, D. Voehringer;*
Department of Infectionbiology, Erlangen, Germany.

Introduction: Dendritic cells (DCs) play an important role as antigen-presenting cells for T-cells, but they can also induce T-cell tolerance and protect against autoimmunity. We recently reported the generation of constitutively DC-deficient mice (ΔDC mice) which can be used to address the specific function of DCs in vivo. These mice lack >95% of classical DCs, the majority of plasmacytoid DCs and Langerhans cells. ΔDC mice show impaired negative thymic selection, hyperimmunoglobulinemia and autoantibody production. About 40%of these mice develop severe autoimmune pathology starting at 6-8 weeks of age.
Objectives: We are investigating the cellular mechanisms which lead to inflammation and autoimmunity in ΔDC mice.
Methods and results: We observed that mice lacking DCs have reduced numbers of regulatory T-cells (Treg) in the gut. Microarray and flowcytometry analysis showed that those Treg show a higher expression level of inhibitory surface receptors. Analysis of suppressive function of Treg from ΔDC mice in in vitro assays revealed that Treg from ΔDC mice are less suppressive, but Treg from both strains suppress equally well in an adoptive transfer model of colitis with Rag-ko recipients. Using DC-deficient Rag-ko mice as recipients, we could see that Treg from both strains show impaired function. By creating mixed bone marrow chimeras where DCs lack MHC-II we observed weightloss, intestinal inflammation and an expression pattern of inhibitory molecules on Treg similar to ΔDC mice.
Conclusion: From our studies we can conclude that contact to DCs via MHC-II is required for proper Treg function and prevention of severe autoimmune inflammation.

WS.C1.04.06

Engineering antigen-expressing regulatory T cells to modulate adverse immune responses

D. W. Scott*, M. Abdeladhim, A. Zhang, J. H. Yoon, K. Parvathaneni, L. Kropp, Y. C. Kim, E. Mitre;*
Uniformed Services University of the Health Sciences, Bethesda, United States.

Expanded regulatory cells (Tregs) have been proposed for the treatment of adverse immune responses to biotherapeutics, autoimmunity, transplantation and allergy. To increase efficacy and specificity of Tregs, we previously engineered human and mouse T cells to express chimeric antigen receptors (CARs) by expressing either T-cell receptors (TCR) from human T-cell clones (derived from patients) or specific single chain fragments (scFv). All of those engineered specific Treg cells actively suppressed effector antibody responses *in vitro* and *in vivo* and modulated EAE. Recently, we expanded this approach to create antigen-specific cytotoxic T cells as well as Tregs expressing antigenic domains (B-cell antibody receptors or BARs). Such BARs are able to kill antigen-specific hybridomas and LPS-activated normal B cells *in vitro*. In one model, BAR Tregs expressing clotting Factor VIII (FVIII) actively and specifically suppressed an antibody response to FVIII *in vivo.* BAR Tregs expressing ovalbumin (OVA) modulated the anaphylactic response (as monitored by temperature drops) to OVA. These results provide a novel paradigm to specifically regulate harmful immune responses in patients. (Supported by grants from the NIH)

WS.C2.01 Signaling in autoimmunity

WS.C2.01.01

Induction of gliadin-specific immune inhibition following treatment with Tolerogenic Immune Modifying Nanoparticles (TIMP) containing-gliadin

J. R. Podojil[1,2], *T. L. Freitag*[3], *R. Pearson*[4,2], *D. Getts*[2], *L. D. Shea*[4], *S. D. Miller*[1];
[1]*Northwestern University, Chicago, United States,* [2]*COUR Pharmaceuticals Development Company, Chicago, United States,* [3]*University of Helsinki, Helsinki, Finland,* [4]*University of Michigan, Ann Arbor, United States.*

In celiac disease, tolerance to gluten proteins from cereals is lost. Toleragenic Immune Modifying Nanoparticles (TIMP) are poly(lactide-co-glycolide) that contain autoreactive protein or peptide epitopes. These nanoparticles have been shown to induce immune tolerance in numerous autoimmune conditions. The identification of gliadins as the primary epitopes in celiac disease suggest that TIMP containing gliadin may serve as a tool to induce tolerance to gliadin and potentially cure celiac disease. Here we developed gliaidn containing TIMP, referred to as TIMP-GLIA, and examined its safety and ability to induce immune tolerance in a delayed type hypersensitivity (DTH) and celiac disease animal model setting. The present data determine the safety and preclinical efficacy of TIMP-GLIA to restore gluten tolerance. *In vitro* studies demonstrated TIMP-GLIA were compatible with intravenous infusion in humans, induced a tolerogenic phenotype of human dendritic cells, and did not trigger T cell activation when incubated with PBMCs from active CD patients. In three mouse models of celiac disease, 1) a delayed-type hypersensitivity, 2) a HLA-DQ8 transgenic, and 3) an adoptive gliadin memory T cell transfer model, treatment with TIMP-GLIA resulted in antigen-specific reductions in T cell proliferation, inflammatory cytokine secretion, circulating gliadin-specific IgG/IgG2c, gluten-dependent enteropathy, and body weight loss. The results provide preclinical support for the safety and efficacy of TIMP-GLIA treatment in re-establishing peripheral immune tolerance in CD patients.

WS.C2.01.02

Inhibition of *arginase-1* expression by the transcription factor Fra-1 in macrophages exacerbates rheumatoid arthritis inflammation

N. Hannemann[1], *S. Cao*[1], *A. Schnelzer*[1], *J. Jordan*[2], *M. Eberhardt*[3], *U. Schleicher*[4], *S. Uebe*[5], *A. Ekici*[5], *J. Rech*[1], *T. Bäuerle*[2], *C. Bogdan*[4], *J. Vera*[3], *G. Schett*[1], *A. Bozec*[1];
[1]*Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Department of Internal Medicine 3 – Rheumatology and Immunology, Universitätsklinikum Erlangen, Erlangen, Germany,* [2]*Institute of Radiology, Preclinical Imaging Platform Erlangen (PIPE) Universitätsklinikum Erlangen, Erlangen, Germany,* [3]*Department of Dermatology, Laboratory of Systems Tumor Immunology Universitätsklinikum Erlangen, Erlangen, Germany,* [4]*Friedrich-Alexander-University Erlangen-Nuremberg (FAU), Institute of Microbiology – Clinical Microbiology, Immunology and Hygiene, Universitätsklinikum Erlangen, Erlangen, Germany,* [5]*Institute of Human Genetics Universitätsklinikum Erlangen, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany.*

The activator protein (AP)-1 transcription factor family, especially its subfamily of FOS proteins (cFos, FosB, Fra-1 and Fra-2), are associated to the regulatory network of macrophage responses. Macrophages are central player during rheumatoid arthritis (RA). This study aims to delineate the role of Fra-1 in macrophages during the acute destructive inflammatory phase of RA. Therefore, we applied the serum-induced arthritis (K/BxN) model to Fra-1 deficient mice controlled by the Mx1 promoter (Fra-1$^{\Delta Mx}$) or the LysM promoter (Fra-1$^{\Delta LysM}$). Fra-1 mutant mice had decreased arthritis severity compared to their littermate wildtype mice. The alleviated arthritis was accompanied to increased arginase-1 (Arg1) expression and activity in the joints, suggesting that its anti-inflammatory features milder RA inflammation. Sorting of immune cell populations revealed macrophages as the major source of Arg1, which was increased in Fra-1 mutant mice. Mechanistically, chromatin immunoprecipitation (ChIP) sequencing and conventional ChIP, as well as luciferase functional reporter analysis uncovered, that Fra-1 transcriptionally inhibited Arg1 expression in macrophages. Moreover, inhibition of Arginase in Fra-1 mutant mice restored a full blunt inflammatory RA response and the supplementation of mice with L-arginine, leading to increased arginase activity in the joint, is sufficient to milder arthritis. Synovium histological sections from RA patients showed a correlation between Arg1, Fra-1 and the DAS28 score, confirming that increased Arg1 activity is of benefit also for human inflammatory joint disease. Our data show for the first time that Fra-1 is a pivot between pro- and anti-inflammatory macrophage. By inhibiting Arg1 activity, Fra-1 exacerbates RA inflammation and joint destruction.

WS.C2.01.03

OPAL1, a novel transmembrane adaptor protein regulates CXCR4 signalling

Š. Borna, *A. Drobek, J. Králová, M. Fabišik, T. Brdička;*
Institute of Molecular Genetics, Prague 4, Czech Republic.

Spatial distribution of immune cells in the body of vertebrates is essential for proper function of the immune system. Destination of each cell is defined by gradient of chemokines, composition of extracellular matrix, and expression pattern of surface receptors, of which chemokine receptors play the most critical role. We have analysed the function Outcome Predictor of Acute Leukemia 1 (OPAL1) a transmebrane adaptor protein with unknown function that is upregulated in TEL/AML1-positive childhood acute lymphoblastic leukemia and higher expression of which was suggested to be associated with favourable prognosis. Using shRNA-mediated knock downs we found that it is a negative regulator of CXCR4 signalling in immortalized murine monocyte progenitors and in human TEL/AML1-positive leukemic B cell line REH. Moreover, OPAL1 knock down in zebrafish changed the organisation of immune cells in the embryo. However, our analysis of OPAL1-deficient mice revealed rather mild phenotype, which is inconsistent with enhanced CXCR4 signalling. Moreover, both splenocytes and bone marrow cells showed normal response to CXCL12, a ligand of CXCR4. We speculated that over time these mice can compensate for the loss of OPAL1 function. In accordance with our hypotheses, bone marrow cells from inducible OPAL1 knock out mice have enhanced CXCR4 signalling upon acute OPAL1 deletion. Our studies of the molecular mechanism show that OPAL1 interacts with Nedd4 family ligases, known regulators of CXCR4 signalling, and suggest that through these interactions OPAL1 modifies CXCR4 signalling.
This study was supported by GACR project 16-07425S.

WS.C2.01.04

FcγR-TLR cross-talk promotes inflammation by human antigen presenting cells via IRF5-dependent gene transcription and glycolytic reprogramming

W. Hoepel[1,2], *L. T. Vogelpoel*[1,2], *I. S. Hansen*[1,2], *M. L. Kapsenberg*[1,2], *D. L. Baeten*[1,2], *B. Everts*[3,4], *J. den Dunnen*[1,2];
[1]*University of Amsterdam, Amsterdam, Netherlands,* [2]*Academic Medical Center, Amsterdam, Netherlands,* [3]*University of Leiden, Leiden, Netherlands,* [4]*Leiden University Medical Center, Leiden, Netherlands.*

Antigen presenting cells (APCs) are crucial for initiation of adequate inflammatory responses, which critically depends on the cooperation of different receptors. An important recently identified route of induction of inflammation by APCs involves cross-talk between Toll-like receptors (TLRs), recognizing microbial structures or dead/damaged host cells, and low-affinity Fc gamma receptors (FcγRs), recognizing IgG immune complexes. The physiological function of this FcγR-TLR cross-talk is to provide protective immune responses against invading pathogens. However, undesired activation of FcγR-TLR cross-talk, e.g. by autoantibodies, also plays a major role in the development of chronic inflammatory disorders such as rheumatoid arthritis (RA). Since interfering with FcγR-TLR cross-talk may have great therapeutic potential, we set out to identify the responsible molecular mechanisms. Strikingly, we identified that production of pro-inflammatory cytokines by FcγR-TLR cross-talk critically depends on activation of interferon regulatory factor 5 (IRF5), which amplifies gene transcription and induces metabolic reprogramming. We show that TLRs and FcγR synergize by inducing two independent signaling pathways that ultimately converge on IRF5 activation. First, TLR stimulation resulted in phosphorylation of TBK1/IKKε, which is required for IRF5 phosphorylation and subsequent activation. Second, we identified that FcγR stimulation signals via a Syk-dependent pathway to induce IRF5 nuclear translocation for amplification of gene transcription. Additionally, FcγR stimulation strongly increases the glycolytic rate of APCs, which is also essential for the synergistic induction of inflammation by FcγR-TLR cross-talk. Taken together, these data provide new potential targets to suppress inflammation in auto-antibody associated diseases such as RA, systemic sclerosis, and systemic lupus erythematosus (SLE).

WS.C2.01.05

Transmembrane TNF signaling through TNF-RI induces SpA-like inflammation, whereas signaling through TNF-RII is crucial for new bone formation

M. van Tok[1], *D. Pots*[1], *I. Blijdorp*[1], *M. Armaka*[2], *G. Kollias*[3], *M. van de Sande*[1], *D. Baeten*[1], ***L. van Duivenvoorde***[1];
[1]*Amsterdam Rheumatology and immunology Center, Amsterdam, Netherlands,* [2]*Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece,* [3]*Biomedical Sciences Research Center "Alexander Fleming",, Vari, Greece.*

Background. TNF can drive strictly distinct inflammatory pathologies depending on its expression form. We have shown that transmembrane (tm)TNF rather than soluble TNF contributes to key pathological features of spondyloarthritis (SpA), including the key hallmark pathological new bone formation. **Objective.** Delineate the cellular and molecular mechanisms by which selective tmTNF overexpression leads to SpA-like pathology. **Methods.** tmTNFtg mice (TgA86) were crossed with TNF-RI or TNF-RII knock-out mice and followed clinically for SpA development. Calvarial fibroblasts were cultured and differentiated towards osteoblasts. **Results.** SpA was observed in all tmTNF$^{+/WT}$ and tmTNF$^{+/WT}$xTNF-RII$^{-/-}$ mice but not in tmTNF$^{+/wt}$xTNF-RI$^{-/-}$ mice and confirmed by histology. Whereas this indicates that TNF-RI is required for tmTNF-induced inflammation, it was striking that 50% of the tmTNF$^{+/WT}$ versus none of the tmTNF$^{+/WT}$xTNF-RII$^{-/-}$ mice depicted clear histological signs of endochondral new bone formation. To test whether TNF-RII is involved in new bone, calvarial fibroblasts from tmTNF$^{+/WT}$, tmTNF$^{+/WT}$xTNF-RI$^{-/-}$, tmTNF$^{+/WT}$xTNF-RII$^{-/-}$ and wild type mice were differentiated with osteogenic medium with or without IL-17A. tmTNF overexpressing fibroblasts enhanced osteogenic differentiation as observed by alkaline phosphatase and alizarin red staining and increased mRNA levels of Collagen type I and ALP compared to wild type fibroblasts. This enhancement in osteogenesis was maintained in tmTNF$^{+/WT}$xTNF-RI$^{-/-}$-derived fibroblasts but abolished in tmTNF$^{+/WT}$xTNF-RII$^{-/-}$-derived fibroblasts. **Conclusion.** The SpA-like phenotype in tmTNFtg mice is crucially dependent on TNF-RI to drive inflammation, but TNF-RII signaling is required for new bone formation under inflammatory conditions.

WS.C2.01.06
Lactate, via SLC5A12, lights up inflammation in CD4+ T cells by inducing a metabolic reprogramming

***V. Pucino**, M. Certo, D. Cucchi, M. Lewis, K. Goldmann, M. Bombardieri, C. Pitzalis, C. Mauro;*
William Harvey Research Institute, Queen Mary, University of London, London, United Kingdom.

Introduction: Inflammatory-sites are characterised by the accumulation of lactate. CD4+ T-cells sense lactate, via the transporter SLC5A12, which in turn inhibits their motility and promotes their switch to the Th17 subset. We assessed whether the lactate/SLC5A12-induced-metabolic-signalling-pathway is key to the chronic inflammation and autoimmunity.
Material and methods: Mononuclear-cells were isolated from tonsil of patients undergoing tonsillectomy from peripheral blood of healthy volunteers and from peripheral blood and synovial fluid of RA-patients. SLC5A12 expression was evaluated by flow-cytometry. RNA-sequencing-analysis was performed for the expression of metabolic-genes in synovial tissues of naïve-to-treatments RA-patients. IL17 expression was assessed by RT-PCR and ELISA. Seahorse and western-blot analysis were performed for the evaluation of metabolic pathways. IL17-signalling-pathway was evaluated by western-blot.
Results: SLC5A12 is up-regulated by CD4+ but not CD8+ T-cells upon triggering of the T-cell receptor (TCR). This expression is higher in CD4+ T-cells isolated from RA synovial fluid, where lactate is more abundant, compared to peripheral RA and healthy donors CD4+ T-cells. Lactate-uptake by CD4+ T-cells through SLC5A12 causes a reprogramming of glycolysis and fatty acids metabolism that tied to migration and cytokine responses via the induction of specific signalling pathways. Antibody-mediated blockade of SLC5A12 limits lactate induced CD4+ T-cells IL-17A and restores lactate-impaired CD4+ T-cell migration by rescuing glycolysis hence leading to beneficial egression of inflammatory CD4+ T-cells from inflamed tissues. In vivo treatment with SLC5A12 blocking antibody ameliorates the clinical course of disease in a mouse model of arthritis.
Conclusions: Targeting lactate/SLC5A12-induced-metabolic-signalling-pathway may provide a novel therapeutic-strategy to reduce inflammation.

WS.C2.02 Neuroinflammatory disorders

WS.C2.02.01
Efficient suppression of effector T cells isolated from multiple sclerosis patients by autologous, UniCAR-engrafted Tregs

***A. Kegler**[1], S. Koristka[1], C. Arndt[1], A. Feldmann[1], S. Albert[2], G. Ehninger[3,4,5], M. Bornhäuser[3,4,5], M. Schmitz[6,4,5], K. Akgün[7], T. Ziemssen[7], M. Bachmann[1,2,4];*
[1]Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Institute of Radiopharmaceutical Cancer Research, Dresden, Germany, [2]UniversityCancerCenter (UCC) 'Carl Gustav Carus' TU Dresden, Tumor Immunology, Dresden, Germany, [3]Medical Clinic and Policlinic I, University Hospital 'Carl Gustav Carus', TU Dresden, Dresden, Germany, [4]German Cancer Consortium (DKTK), partner site Dresden; and German Cancer Research Center (DKFZ), Heidelberg, Germany, [5]National Center for Tumor Diseases (NCT), Dresden, 'Carl Gustav Carus' TU Dresden, Dresden, Germany, [6]Institute of Immunology 'Carl Gustav Carus' TU Dresden, Dresden, Germany, [7]Center of Clinical Neuroscience, Department of Neurology, University Hospital ´Carl Gustav Carus', TU Dresden, Dresden, Germany.

Introduction: In multiple sclerosis (MS) patients pathogenic, autoreactive effector T cells (Teffs) provoke demyelination and central nerve system damage. To impede those harmful immune reactions, the adoptive transfer of regulatory T cells (Tregs) emerged as a promising therapeutic strategy. Several preclinical mouse models confirm an inferior functionality of polyclonal compared to antigen-specific Treg cells. However, isolation and expansion of Tregs with a desired antigen-specificity proves to be highly time-consuming and labor-intensive. Materials and Methods: To overcome these hurdles, we armed polyclonal Tregs isolated from MS patients with a universal chimeric antigen receptor (UniCAR) construct. This innovative technology allows a site-specific redirection of cells against any desired surface structure, as cross-linkage to target cells is mediated by a separate, antigen-binding targeting module (TM). Results: Highly pure $CD4^+CD25^{high}CD127^{dim}CD45RA^+$ MS-Tregs could be genetically modified to stably express the UniCAR 4-1BB/ζ construct. UniCAR-endowed Tregs strongly expand and show phenotypic stability also upon pro-inflammatory challenge. By adding a TM in the presence of target cells, UniCAR-engrafted Tregs are antigen-specifically activated demonstrated by CD69 and LAP upregulation. Most importantly, upon TM-stimulation UniCAR-armed Tregs efficiently suppress pre-activated, patient-derived Teffs. Conclusions: Taken together, the UniCAR system holds an enormous therapeutic potential for MS, as it not only allows a site-specific and precisely regulated Treg activation but also confers strong suppressive capacity to Tregs from MS patients. Thereby, this innovate technology might broaden current treatment strategies to overcome impaired functionality of Tregs as well as resistance of pathogenic Teffs to Treg suppression reported for MS patients.

WS.C2.02.02
elucidation of pro-resolving lipid mediators in the cerebrospinal fluid: implications for multiple sclerosis pathogenesis

***G. Kooij**[1,2], V. Chiurchiù[3], G. Enzmann[4], P. Norris[2], M. Khameni[5], B. Engelhardt[4], H. E. de Vries[1], C. Serhan[2];*
[1]VUmc MS Center Amsterdam, Amsterdam Neuroscience, Amsterdam, Netherlands, [2]Harvard Medical School, Boston, United States, [3]European Center for Brain Research, Rome, Italy, [4]Theodor Kocher Institute, Bern, Switzerland, [5]Karolinska Institutet, Stockholm, Sweden.

Background and objective The acute inflammatory response is host protective and efficient resolution of inflammation is required to prevent excessive inflammation and restore tissue homeostasis. This protective process is orchestrated by specialized pro-resolving lipid mediators (SPMs) that are biosynthesized from omega-3 fatty acids. In the chronic neuro-inflammatory disease multiple sclerosis (MS), the abundant presence of pro-inflammatory cells and wide-spread microglial activation within the central nervous system (CNS) suggests that this resolution process is impaired. Consequently, the uncontrolled inflammatory response will acquire a chronic nature, leading to severe tissue damage (neurodegeneration) and disease progression. To date, fundamental insights into the regulation of CNS resolution processes and whether impairments in this system correlate with MS progression remain elusive.
Methods We used liquid chromatography-tandem mass spectrometry (LC-MS-MS)-based metabololipidomics to reveal lipid mediator signatures in the cerebrospinal fluid (CSF) of RRMS (either in relapse or remission), SPMS and PPMS patients as well as age/sex matched controls.
Results Here we provide first evidence for resolution of inflammation defects in MS as reflected by a complete absence of brain specific SPMs in the CSF in all MS cases. We also defined the source of these SPMs, their target cells in the CNS during neuro-inflammation and determined their *in vivo* efficacy in an MS mouse model.
Conclusion By using metabololipidomic profiling of human CSF, we here provide first evidence that impaired resolution pathways exists in MS and thereby highlight the potential to use SPMs as novel therapeutics as well as biomarkers for diagnosis.

WS.C2.02.03
Characterizing human CD20+ T cells and their role in multiple sclerosis

***M. von Essen**[1], C. Ammitzbøll[1], R. Hansen[1], O. McWilliam[1], E. Petersen[1], H. Marquart[2], F. Sellebjerg[1];*
[1]Danish Multiple Sclerosis Center, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, [2]Department for Clinical Immunology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark.

Introduction: Recently we found that T cells expressing CD20 showed high reactivity to central nervous system (CNS) antigens. In addition, the frequency of CD20+ T cells was increased in blood of patients with multiple sclerosis (MS), suggesting a role in the pathogenesis. In this study, we further characterized the phenotype of CD20+ T cells and their implication in MS.
Results: Analysing the expression of chemokine receptors and adhesion molecules, assumed to recruit blood T cells to the CNS, showed that the frequency of blood CD20+ T cells from patients with relapsing-remitting MS (RRMS) expressing CCR2, CCR5, CCR6, CXCR3 and a high level of CD49d was significantly increased compared to CD20- T cells. This increased CNS migration potential was substantiated by the observation that CD20+ T cells were enriched in the cerebrospinal fluid (CSF) from patients with RRMS. A role in MS pathogenesis was further supported by a positive correlation between CD20+ T cells in the CSF and demyelination measured as free myelin basic protein in the CSF as well as a positive correlation with disease severity (EDSS). Characterizing CD20+ T cells revealed a pro-inflammatory T cell producing high levels of IFN-γ, TNF-α and GM-CSF as well as granzyme A and K compared to CD20- T cells; both in the CD4 and the CD8 compartment.
Conclusions: Our data indicate that CD20+ T cells have a Th1/Tc1 phenotype and possibly cytolytic activity and that they may play a central role in the pathogenesis of MS.

WS.C2.02.04

Malat-1 LncRNA regulates inflammation and T cell differentiation in an animal model of multiple sclerosis

***F. Masoumi**, S. Ghorbani, F. Talebi, F. Noorbakhsh;*
Immunology Department, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of.

BACKGROUND: A growing body of evidence points to the role of long noncoding RNAs (lncRNAs) in the pathogenesis of neurological diseases. Nonetheless, our knowledge of multiple sclerosis-related lncRNAs remains limited. Herein, we investigated the potential role of Malat-1 lncRNA in the context of autoimmune neuroinflammation. **METHODS:** The expression level of Malat-1 was measured in CNS tissues from EAE mice as well as stimulated splenocytes and macrophages using qPCR. To examine the role of Malat-1 in macrophages polarization, Malat-1 siRNA was transfected into primary macrophages followed by M1/M2 macrophage polarization. The expression of M1/M2 markers were then evaluated. Also, the role of the Malat-1 in T cell differentiation was investigated by transfection of CD4+ T cells with Malat-1 siRNA, followed by intracellular cytokine staining. Moreover, effect of Malat-1 downregulation on T cell proliferation was investigated using CFSE staining. **RESULTS:** Expression of Malat-1 was significantly decreased in the spinal cords of EAE mice at days 15 and 25 post disease induction. Stimulated splenocytes showed significant upregulation of Malat-1, whereas expression of Malat-1 in activated macrophages was reduced. Malat-1 downregulation enhanced macrophages polarization towards M1 phenotype. Also, Malat-1 downregulation in activated lymphocytes shifted the pattern of T cell differentiation towards Th1 and Th17 cells while differentiation towards Tregs was decreased. Besides, T cell proliferation was increased following Malat-1 downregulation. **CONCLUSION:** Our data highlight the anti-inflammatory actions of Malat-1 in the context of autoimmune neuroinflammation. Malat-1 influences differentiation of T cells and activation of macrophages, providing potential therapeutic options for controlling inflammation in MS.

WS.C2.02.05

Dimethyl fumarat limits Tc17 cell fate in autoimmunity via ROS accumulation

***C. Lückel**[1], H. Raifer[2], L. Campos Carrascosa[3], S. Moos[4], F. Kurschus[4], F. Marini[5], M. Klein[1], T. Bopp[1], Y. Chao[6], C. Zielinski[6], L. Bonetti[7], M. Grusdat[7], D. Brenner[7], B. Tackenberg[8], M. Huber[2];*
[1]Institute for Immunology, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany, [2]Institute for Medical Microbiology and Hospital Hygiene, University Marburg, Marburg, Germany, [3]Laboratory of Gastroentrology and Hepatology, Erasmus MC University Medical Center, Rotterdam, Netherlands, [4]Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany, [5]Institute for Medical Biostatistics, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany, [6]Institute forMedical Microbiology, Immunology and Hygiene, Technical University of Munich, Munich, Germany, [7]Department of Infection and Immunity, Experimental and Molecular Immunology, Luxembourg, Luxembourg, [8]Center of Neuroimmunology, University of Marburg, Marburg, Germany.

Introduction: Dimethyl fumarate (DMF) is approved for treatment of relapsing remitting multiple sclerosis (RRMS), a chronic inflammatory disease of the central nervous system (CNS) that is mediated by autoreactive T cells. The enrichment of IL-17-producing $CD8^+$ T (Tc17) cells in cerebrospinal fluid of MS patients and the co-pathogenic function during EAE point to their contribution to CNS autoimmunity in men and mice. So far, a direct impact of DMF on $CD8^+$ T cells is unknown.
Material and Methods: Analysis of human and murine Tc17 cells and quantification of ROS levels were determined by flow cytometry. To examine changes on transcriptome, RNA-Seq and bioinformatical analysis (GSEA, PCA) were performed. Chromatin immunoprecipitation and Western Blot were conducted to evaluate histone modifications.
Results: We revealed that DMF upregulated reactive oxygen species (ROS) in Tc17 and Th17 cells by glutathione depletion, leading to IL-17 suppression preferentially in Tc17 cells. Accordingly, IL-17 production of $CD8^+$ but not of $CD4^+$ T cells was reduced in DMF-treated MS patients and DMF abrogated Tc17 cell pathogenicity in a mouse model. Accumulated ROS shifted Tc17 cells towards cytotoxic T lymphocyte signature by enhancing PI3K-AKT and STAT5 pathways along with altered histone modifications at the *Il17* locus. AKT deactivated FOXO1 leading to the upregulation of T-bet, which in turn suppressed IL-17. In line, T-bet-deficiency, inhibition of HDACs, PI3K-AKT or STAT5 prevented DMF-mediated suppression of Tc17 cells.
Conclusions: Our data provide mechanistic insights into selective modulation of Tc17 cell fate by upregulated ROS and IL-2 signaling with relevance for IL-17-driven pathologies.

WS.C2.02.06

The TH17-associated cytokine interleukin-26 regulates central nervous system barrier function

***B. Broux**[1], E. Peelen[2], E. Gowing[2], S. Zandee[2], M. Charabati[2], M. Lécuyer[3], L. Hachehouche[2], L. Bourbonnière[2], C. Pittet[2], S. Larouche[2], J. I. Alvarez[4], A. Bouthillier[5], R. Moumdjian[5], P. Duquette[2], N. Arbour[2], A. Prat[2];*
[1]Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium, [2]CRCHUM, Montreal, Canada, [3]University Medical Centre, Goettingen, Germany, [4]University of Pennsylvania, Philadelphia, United States, [5]CHUM, Montreal, Canada.

In multiple sclerosis (MS), demyelinated lesions are caused by an inflammatory response in the central nervous system (CNS). Pathogenic T helper (T_H)17 $CD4^+$ T lymphocytes are involved in the development of these lesions. Two cytokines expressed by these cells, namely IL17 and IL22, have been shown to disrupt blood brain barrier (BBB) integrity, an important early event in MS lesion formation. Here, we report that IL26 expression is induced in human $CD4^+$ T lymphocytes by T_H17-inducing cytokines and is significantly upregulated in the blood and cerebrospinal fluid of MS patients. In addition, $CD4^+IL26^+$ T lymphocytes are found in perivascular immune infiltrates in MS brain lesions and the two receptor chains for IL26, IL10R2 and IL20R1 are detected on BBB endothelial cells (ECs) *in vitro* and *in situ*. Unexpectedly, we found that IL26 promotes tightness and reduces permeability of BBB-ECs *in vitro* and *in vivo*. Finally, treatment of mice with experimental autoimmune encephalomyelitis (EAE; an animal model of MS) with IL26 reduces disease severity and pro-inflammatory lymphocyte infiltration into the CNS. Our study demonstrates that although IL26 is largely a T_H17-associated cytokine, it promotes BBB integrity *in vitro* and *in vivo,* and using it as a therapy induces amelioration of neuroinflammation.

WS.C2.03 Pathophysiology of autoimmune diseases

WS.C2.03.01

Cholesterol metabolism drives the function of regulatory B cells

***J. Bibby**, A. Cope, E. Perucha;*
King's College London, London, United Kingdom.

Across a life cycle, immune cells transition between vastly different metabolic profiles. This differing reliance on specific metabolic pathways dictates fundamental cellular processes, such as activation and functional output. However, despite its critical importance to immune homeostasis – as patients with mutations in the cholesterol biosynthesis pathway (CBP) enzyme mevalonate kinase, present with autoinflammatory disease – lipid metabolism has been relatively understudied.
Here were describe a central role for the CBP in regulatory B cell function. Initial observations showed that inhibition of the CBP specifically blocks the expression of IL-10 in primary human B cells. Fitting with this observation, perturbation of the pathway renders B cells unable to suppress CD4 T cell responses. Mechanistically, the CBP is shown to regulate IL-10 induction through its driving of protein isoprenylation. This is achieved through the CBP metabolic intermediate geranylgeranyl diphosphate, which, through catalytic addition of this isoprenoid group, regulates the localisation of signalling proteins. This isoprenylation event is shown to be critical in permitting signalling through the TLR9-PI3K-Akt-GSK3 pathway in human B cells, which is necessary for IL-10 expression. Finally, RNA sequencing analysis revealed that protein isoprenylation drives the expression of known transcription factors that regulate IL-10 expression.
These data uncover a novel and central role for cholesterol metabolism in human regulatory B cells. The production of geranylgeranyl diphosphate by the CBP is shown to specifically orchestrate the expression of the effector cytokine IL-10, whilst simultaneously leaving inflammatory cytokine production unperturbed.

WS.C2.03.02

Neutrophil activation and altered coagulation in a rat model for autoimmune hemolytic anemia

***L. Del Vasto Nunez**[1], G. van Mierlo[1], I. Jongerius[1], B. M. Luken[1], N. Juffermans[2], S. Zeerleder[1,2];*
[1]Sanquin Research, Amsterdam, Netherlands, [2]Academic Medical Center, Amsterdam, Netherlands.

Autoantibodies against red blood cells (RBCs) in autoimmune hemolytic anemia (AIHA) may lead to complement activation and intravascular hemolysis with subsequent release of toxic cell-free heme. AIHA patients with intravascular hemolysis and complement activation have a higher risk of thrombosis. Complement C5a and heme trigger activation of neutrophils and, as shown recently, neutrophil activation and formation of Neutrophil extracellular traps (NETs) are closely linked to thrombosis. We recently found decreased neutrophil activation in AIHA patients after complement inhibitor treatment. We therefore hypothesized that complement-mediated neutrophil activation and the formation of pro-thrombotic NETs occur in AIHA and contribute to hypercoagulability. We aimed to design a xenograft transfusion model in rats to induce antibody-initiated complement activation and intravascular hemolysis mimicking the pathophysiology of AIHA and study the role of neutrophils in the pathogenesis of thrombosis. Male Wistar rats were subjected to a transfusion with human RBCs followed by multiple blood draws. Markers for complement and neutrophil activation and signs of hypercoagulability were determined at each time point. Transfused rats exhibited increased plasma C4b/c, elastase and nucleosome levels compared to control rats. Next, heme oxygenase 1 (HO-1) mRNA expression was higher in neutrophils from transfused rats than in those from control animals. Transfusion resulted in elevated thrombin generation and plasma thrombin-antithrombin complexes in animals administered human RBCs versus control group. These data suggest that our rat model of AIHA is suitable to induce complement-mediated intravascular hemolysis and the subsequent activation of neutrophils to study the pathogenesis of hemolysis-associated thrombosis.

WS.C2.03.03

JMJD3/UTX histone H3K27 demethylases regulate metabolic and inflammatory Th17 cell function in ankylosing spondylitis

A. P. Cribbs[1], *M. Philpott*[1], *R. K. Prinjha*[2], *P. Wordsworth*[1], *P. Bowness*[1], *M. Feldmann*[3], *U. Oppermann*[1];
[1]*Botnar Research Centre, Oxford, United Kingdom,* [2]*Epinova Discovery Performance Unit, GSK, Stevenage, United Kingdom,* [3]*Kennedy institute of Rheumatology, Oxford, United Kingdom.*

Introduction: T helper 17 (Th17) cells are CD4+ effector T cells that play an instrumental role in driving pro-inflammation IL-17 production in several autoimmune conditions, such as ankylosing spondylitis (AS).
Aim: Our aim is to identify epigenetic pathways and mechanisms that inhibit Th17 pro-inflammatory cell function. To this end, we use chemical and shRNA knockdown tools to study "readers, writers and erasers of the histone code" that regulate gene transcription.
Results: A focused epigenetic compound screen, comprising 60+ selective epigenetic inhibitors, identified Jumonji-type histone demethylases as playing a fundamental role in regulating Th17 cell inflammatory function. Specifically, using flow cytometry, we showed that inhibition of the JMJD3/UTX demethylases leads to suppression of IL-17 cytokine levels. Transcriptomic analysis revealed a pronounced upregulation of the ATF4 family of transcription factors. We also observed reduced proliferation, which was driven by the ATF4 metabolic stress response, in both healthy donors and patients with AS. Chemical inhibitors of JMJD3/UTX demethylases were overtly impactful on the cellular biochemistry of Th17 differentiated T-cells, with prominent effects on differentiation-related changes in glucose utilization, anabolic processes, and remarkable changes in amino acid and TCA cycle metabolism. Using ChIP-seq and ATAC-seq, we found that this phenotype was driven through global increases in the repressive H3K27me3 histone mark.
Conclusion: We show that inhibition of JMJD3/UTX histone demethylases in Th17 cells leads to an ATF4 metabolic stress response, induction of cellular anergy and a reduction in pro-inflammatory function. Therefore, JMJD3/UTX inhibition may be a tractable therapeutic target in autoimmunity.

WS.C2.03.04

Immunoglobulin A activated myeloid cells induce pathological osteoclast activation in rheumatoid arthritis patients

M. M. J. van Gool*, A. C. Breedveld, R. E. Mebius, M. van Egmond;*
VU University Medical Center, Amsterdam, Netherlands.

Immunoglobulin A (IgA) is crucial for maintaining homeostasis at mucosal sites. However, it is becoming clear that excessive activation of myeloid cells by IgA via FcαRI leads to severe inflammation and tissue damage. Recently, our lab has shown that neutrophils get activated by IgA rheumatoid factor (RF) present in the serum and synovial fluid of rheumatoid arthritis (RA) patients. Since IgA RF is correlated with severe bone erosions in RA patients, we hypothesize that IgA contributes to the pathology of RA. Here we show that cellular functions and cytokine release by IgA activated neutrophils and monocytes results in a specific and profound pro-inflammatory response. Only IgA induces migration of neutrophils, followed by monocytes. Moreover, the release of TNF-α by both cells, IL-6 for monocytes and IL-8 for neutrophils was only seen after IgA activation. To determine the effect of IgA activated cells on osteoclast activation, we performed a bone resorption assay in which osteoclasts were cultured in the presence of supernatant of IgA or IgG activated neutrophils and monocytes. Bone resorption was significantly increased when osteoclasts were cultured in the presence of supernatant of IgA activated cells compared to IgG. This suggests that myeloid cells activated by IgA autoantibody complexes play a role in inducing joint damage in RA patients, hereby increasing disease severity. Moreover, we discovered that differentiated osteoclasts also express FcαRI in contrast to Fcγ receptors, therefore osteoclasts can be directly activated by IgA complexes. Blocking FcαRI may represent a novel therapeutic strategy for the treatment of RA.

WS.C2.03.05

Anti-Ly9 (CD229) antibody treatment reduces marginal zone B cell numbers and salivary gland inflammation in a mouse model of Sjögren's Syndrome

J. Puñet-Ortiz*, M. Sáez Moya, M. Cuenca, A. Lázaro, J. Boix Nebot, P. Engel;*
Universitat de Barcelona, Barcelona, Spain.

Sjögren's Syndrome (SS) is one of the most common chronic autoimmune rheumatic diseases. Characterized by hyper B-cell activation, lymphocyte cell infiltration and tissue damage of exocrine glands, it can also present life-threating extraglandular affectations, such as respiratory/hepatic dysfunction, chronic infections and marginal zone B-cell lymphoma. Several biologic agents have been tested in SS but none has shown significant efficacy. Here we report the effects of antibodies against Ly9 (CD229), which is a cell surface molecule that belongs to the SLAM family of immunomodulatory receptors, using NODH2^{h4} female mice as a model of SS-like disease. Female mice were treated with anti-Ly9 antibody or isotype control at week 24, when all mice present SS-related autoantibodies, salivary gland infiltrates and marginal zone (MZ) B cell pool enlargement. Plasma was collected before and after treatment, and quantified by ELISA and immunofluorescence. B and T lymphocyte subsets from lymphoid tissues and salivary glands were analyzed by flow cytometry. Salivary glands were also studied in paraffin-embedded sections. Autoantibody levels (anti-ANA, anti-Ro, anti-dsDNA and RF) were decreased or impeded to increase over time after anti-Ly9 treatment. Moreover, this treatment induced the depletion of key lymphocyte subsets involved in SS pathology such as MZ and germinal center B cells in the spleen, draining lymph nodes and salivary glands. Importantly, mice receiving anti-Ly9 mAb showed a reduction of the infiltrate within salivary glands (isotype control: 0.61 mm^2 versus anti-Ly9: 0.15 mm^2). These data indicate that Ly9 is a potential therapeutic target for the treatment of SS.

WS.C2.03.06

Dysregulated expression of RasGRP1 in T cells leads to autoimmunity

T. Douma[1], *D. Simeonov*[2], *D. Myers*[2], *M. Baars*[1], *G. Mijnheer*[3], *F. van Wijk*[3], *A. Marson*[2], *J. Roose*[2], ***Y. Vercoulen***[1];
[1]*Center for Molecular Medicine, UMC Utrecht, Utrecht, Netherlands,* [2]*UCSF, San Francisco, United States,* [3]*Laboratory Translational Immunology, UMC Utrecht, Utrecht, Netherlands.*

Introduction: RasGRP1 is a Ras guanine nucleotide exchange factor (GEF), and an essential regulator of lymphocyte receptor signaling. Aberrant expression of RasGRP1 results in defective positive thymocyte selection in mice. Furthermore, recent case reports describe RasGRP1 deficient patients that suffer from recurrent infections and autoimmunity. It is unknown how RasGRP1 levels are regulated and how aberrant expression contributes to autoimmunity.
Results: Increased expression of RasGRP1 directly results in increased Ras-MAPK signaling in lymphocytes. We detected antinuclear antibodies in the serum of rasgrp1$^{+/-}$ and rasgrp1$^{-/-}$ C57/B6 mice, suggesting that loss of RasGRP1 expression is causal for autoimmunity. In patients with juvenile idiopathic arthritis we detected decreased levels of rasgrp1 in CD4$^+$ T cells isolated from synovial fluid, compared to peripheral blood. Next, we analyzed H3K27 acetylation profiles and identified 2 enhancer regions of the RasGRP1 gene, active in CD4$^+$ T cells, with single nucleotide polymorphisms for autoimmune disease. Chip-seq analysis shows binding of T cell lineage transcription factors FOXP3, Tbet, GATA3 to the enhancer region. CRISPR-Cas9 editing of the enhancer resulted in decreased RasGRP1 protein expression levels.
Conclusions: We identified an enhancer region upstream of rasgrp1 that is required for regulation of rasgrp1 expression, and SNPs in this region are associated with autoimmunity. Furthermore, our data reveal that decreased expression of RasGRP1 results in autoimmunity in mice, and that rasgrp1 expression is decreased in T cells in the synovial fluid of patients with juvenile idiopathic arthritis. Together, we show that proper regulation of RasGRP1 expression is essential to prevent inflammatory disease.

WS.C2.04 Therapy of autoimmune disorders

WS.C2.04.01

Functional reprogramming of regulatory T cells in the absence of Foxp3

L. M. Charbonnier[1], *Y. Cui*[1], *D. Lopez*[2], *J. J. Bleesing*[3], *M. I. Garcia-Lloret*[4], *K. Chen*[5], *A. Ozen*[6], *M. O. Li*[7], *T. A. Chatila*[1];
[1]*Boston Children's Hospital and Harvard Medical School, Boston, United States,* [2]*University of California, Los Angeles, United States,* [3]*Cincinnati Children's Hospital Medical Center, Cincinnati, United States,* [4]*David Geffen School of Medicine at the University of California Los Angeles, Los Angeles, United States,* [5]*University of Utah School of Medicine, Salt Lake City, United States,* [6]*Marmara University Faculty of Medicine, Istanbul, Turkey,* [7]*Memorial Sloan Kettering Cancer Center, New York, United States.*

Foxp3-deficient regulatory T (T_{reg}) cells lack suppressor function and manifest a T effector (T_{eff}) cell-like phenotype. We demonstrate that Foxp3 deficiency dysregulates mTORC2 signaling and gives rise to augmented aerobic glycolysis and oxidative phosphorylation. Mutant T_{reg} cell-specific deletion of the mTORC2 adaptor gene *Rictor* or expression of a *Foxo1* transgene improved regulatory function and ameliorated disease. Rictor deficiency reestablished a subset of T_{reg} cell genetic circuits and suppressed the T_{eff} cell-like metabolic program. Treatment of mutant T_{reg} cells of patients with FOXP3 deficiency with mTOR inhibitors similarly antagonized their T_{eff} cell-like program and restored suppressive function. Thus, regulatory function can be reestablished in Foxp3-deficient T_{reg} cells by targeting their metabolic pathways, providing opportunities to restore tolerance in T_{reg} cell disorders.

WS.C2.04.02

Immune response profiling reveals signaling networks mediating TNF-blocker function and predictors of therapeutic responses in spondyloarthritis patients

S. Menegatti[1], *V. Rouilly*[2,3], *E. Latis*[1], *H. Yahia*[1], *E. Mascia*[1], *C. Leloup*[1], *D. Duffy*[2,4], *A. Urrutia*[5], *E. Achouri*[6], *L. Quintana-Murci*[7], *M. Albert*[2,5,4], *C. Miceli*[1,8,9], *M. Dougados*[9,8], *E. Bianchi*[1,9], *L. Rogge*[1,9];
[1]*Institut Pasteur, Immunoregulation Unit, Department of Immunology, Paris, France,* [2]*Institut Pasteur, Center for Translational Research, Paris, France,* [3]*DATACTIX, Paris, France,* [4]*Institut Pasteur, Laboratory of Dendritic Cell Immunobiology, Department of Immunology, Paris, France,* [5]*Genentech Inc, Department of Cancer Immunology, San Francisco, United States,* [6]*Institut Pasteur, Center of Bioinformatics, Biostatistics and Integrative Biology, Paris, France,* [7]*Institut Pasteur, Human Evolutionary Genetics, Department of Genomes & Genetics, Paris, France,* [8]*Paris Descartes University, Rheumatology Department, Cochin Hospital, AP-HP, Paris, France,* [9]*Unité Mixte de Recherche, Institut Pasteur/AP-HP Hôpital Cochin, Paris, France.*

Anti-TNF therapy has revolutionized treatment of several chronic inflammatory diseases. However, its impact on the immune system is incompletely understood and predicting therapeutic responses remains a major challenge since TNF-blockers are effective only in a subpopulation of patients.
We have used whole-blood, syringe-based assays performing ex vivo stimulation (TruCulture assays) to define the impact of anti-TNF therapy on immune responses to microbial challenges and stimuli targeting specific immune pathways in spondyloarthritis patients, and to identify immunological correlates predicting therapeutic responses.
We found that anti-TNF therapy induces specific changes in immune responses of patients to various stimuli. These changes can be measured in stimulated, but not resting immune cells and are detectable already after a single injection of anti-TNF. Quantitative set analysis for gene expression (QuSAGE) of the stimulation cultures revealed that the gene modules most affected by anti-TNF therapy are NFkB-transcription factors and target genes, suggesting that TNF-blockers primarily act by breaking TNF- and IL-1-dependent feed-forward loop of NF-kB activation. We also tested if induced immune responses correlate with therapeutic responses. Using machine-learning algorithms, we found that expression of several molecules regulating key steps of leucocyte migration and invasiveness was significantly higher in patients responding to anti-TNF therapy following LPS-stimulation, while expression of cytotoxic and T-cell genes was higher in non-responders. The random forest model that we trained and validated using 13 selected biomarkers has a predictive power of 83%. We propose that immune response profiling of patients before therapy is a powerful new strategy to help guiding clinical decisions.

WS.C2.04.03

Targeting NF-κB signalling in B cells: a potential new treatment modality for antibody mediated autoimmune diseases

J. P. van Hamburg[1], *P. Tuijnenburg*[2], *B. Helder*[1], *L. van Keep*[1], *K. Wesenhagen*[1], *P. Kucharzewka*[3], *M. H. Jansen*[2], *A. Al-Soudi*[i], *P. L. Klarenbeek*[1], *H. Olsson*[3], *N. de Vries*[1], *T. W. Kuijpers*[2], *S. W. Tas*[1];
[1]*Amsterdam Rheumatology & immunology Center | Department of Experimental Immunology, Academic Medical Center/University of Amsterdam, Amsterdam, Netherlands,* [2]*Department of Experimental Immunology, Academic Medical Center/University of Amsterdam, Amsterdam, Netherlands,* [3]*Respiratory, Inflammation and Autoimmunity IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden.*

B cells play a key role in the pathogenesis of autoimmune diseases such as ANCA-associated vasculitis (AAV). NF-κB signalling can be activated by various B cell receptors, including the B cell antigen receptor, CD40, BAFFR and TLRs, and is crucially involved in B cell responses. In this study, we examined whether targeting of NF-κB signalling by novel pharmacological inhibitors of NF-κB inducing kinase (NIK; non-canonical NF-κB pathway) and IKKβ (canonical NF-κB pathway) is effective in blocking B cell responses. In B cells of AAV patients and healthy donors, targeting of NIK and IKKβ effectively inhibited non-canonical or canonical NF-κB signalling, respectively. In a B cell stimulation assay, NIK and IKKβ inhibition significantly reduced T cell-dependent (anti-IgM+anti-CD40+IL-21) and T cell-independent (CpG+IL-2) B cell proliferation, plasmablast differentiation ($CD27^{++}/CD38^{+}$), and antibody production. The effects of NIK inhibition appeared to be B cell-specific as T cell proliferation was largely unaffected. These data demonstrate that inhibition of NF-κB signalling in B cells results in the modulation of various B cell responses. Ongoing studies will indicate whether targeting of NF-κB signalling in B cells may be an effective novel treatment modality for AAV and other autoantibody-mediated autoimmune diseases.

WS.C2.04.04

Suppression of autoreactive T and B lymphocytes by selective therapy in humanized murine SCID model of systemic lupus erythematosus

S. Bradyanova[1], *N. Mihaylova*[1], *P. Chipinski*[1], *E. Ivanova-Todorova*[2], *D. Kyurkchiev*[2], *M. Herbáth*[3], *J. Prechl*[3], ***A. I. Tchorbanov***[1];
[1]*Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria,* [2]*University Hospital "St. Ivan Rilski", Sofia, Bulgaria,* [3]*Immunology Research Group, Hungarian Academy of Sciences, Budapest, Hungary.*

Introduction: Self-specific B and T cells play a main role in pathogenesis of Systemic lupus erythematosus (SLE) and are a logical target for selective therapy. The complement receptor type 1 (CR1) on human B-lymphocytes has suppressive activity and engagement of this receptor inhibits B cell activation. The protein Annexin A1 (Anx A1), is a modulator of the immune system and abnormal expression was found on activated B and T cells during human autoimmunity. We hypothesize that it may be possible to down-modulate the activity of autoreactive T and B cells from SLE patients in humanized SCID mouse model by treating them with a neutralizing antibody against Anx A1 or by protein engineered molecules, which co-crosslink the BCR and CR1. Materials and Methods: Protein chimeric molecules construction, Immunodeficient SCID mice transfer with human PBMC from SLE patients, ELISA for dsDNA antibodies and cytokines, flow cytometry for apoptosis and activation markers, ELISpot and MTT assays, protein array. Results: Reconstituted SCID mice showed presence of several auto-antibodies, as well as immunoglobulin deposition in the renal glomeruli. Treatment of the transferred SCID mice either with DNA-like chimera and anti-Anx A1 antibody prevented appearance of anti-DNA antibodies and proteinuria, while the PBS-injected animals had high levels after the transfer. The treatment reduced the levels of disease-associated cytokines also. Conclusions: It is possible to down-regulate the activity of pathogenic human T and B cells in humanized SLE-SCID mouse model of SLE by targeting Anx A1 or CR1 with a specific monoclonal antibody or chimeric molecule.

WS.C2.04.05

Interleukin-6 Receptor inhibition, as first-line b-DMARD, affects B cell subpopulations distribution through epigenetic modifications in Rheumatoid Arthritis patients.

C. Di Mario, *B. Tolusso, S. Alivernini, A. Fedele, L. Petricca, M. Gigante, G. Ferraccioli, E. Gremese;*
Fondazione Policlinico Universitario A. Gemelli - Catholic University of the Sacred Heart - Division of Rheumatology, Rome, Italy.

Introduction. B cell maturation is controlled by microRNA-155(miR-155)/PU.1 axis influenced by IL-6 stimulation. The aim of the study was to investigate the effect of IL-6R inhibition on the epigenetic signature of B cells(miR-155/PU.1 axis) in Rheumatoid Arthritis(RA). Methods.Twenty-nine RA starting IL-6R inhibitor were enrolled. At study entry and after 3-6-12-18months follow-up peripheral blood(PB)-derived $CD19^{+}$cells were isolated by magnetic microbeads(Miltenyi) and miR-155 and PU.1 endogenous expression was determined by RT-PCR. Moreover, B cells subpopulations were assessed through FACS analysis (IgD/CD27classification). IL-6 plasma levels was assessed by ELISA. ACR/EULAR criteria were used to assess the response rate to treatment. PB-derived $CD19^{+}$cells of healthy individuals(HC) were used as comparison. Results.At study entry, RA showed higher percentage of $IgD^{-}/CD27^{-}CD19^{+}$cells($p<0.05$) and $IgD^{+}/CD27^{+}CD19^{+}$cells($p<0.05$) than HC. Moreover, $IgD^{-}/CD27^{-}CD19^{+}$cells percentage directly correlated with Disease Activity Score($p=0.04$) and IL-6 plasma levels($p=0.06$). Stratifying patients based on remission achievement, RA who achieved DAS remission under IL-6R inhibition showed a significant decreased of $IgD^{-}/CD27^{-}CD19^{+}$cells percentage compared to not responding patients($p<0.05$), reaching $IgD^{-}/CD27^{-}CD19^{+}$cells percentage comparable to HC($p>0.05$). At baseline, PB-derived $CD19^{+}$cells of RA showed significantly higher endogenous expression of miR-155($p=0.04$) than HC. Moreover, RT-PCR showed that IL-6R inhibition significantly represses endogenous miR-155 expression in PB-derived RA $CD19^{+}$cells after 3 months($p<0.05$) restoring PU.1 expression in PB-derived $CD19^{+}$cells after 6 months($p<0.05$) only in RA achieving disease remission. Conclusions.IL-6R inhibitor acts restoring $CD19^{+}$cells homeostasis through epigenetic modulation in RA repressing endogenous expression of miR-155 in PB-derived $CD19^{+}$cells and restoring PU.1 expression mirrored by the decrease of $IgD^{-}/CD27^{-}CD19^{+}$cells rate in RA achieving disease remission.

WS.C2.04.06

Co-engaging CD47 and CD19 with a bispecific antibody impairs B cell proliferation induced by B-cell receptor cross-linking

E. Hatterer[1], *L. Barba*[1], *N. Noraz*[2], *B. Daubeuf*[1], *J. Aubry-Lachainaye*[3], *B. van der Weid*[1], *F. Richard*[1], *W. Ferlin*[1], *M. Kosco-Vilbois*[1], *V. Buatois*[1], *L. Shang*[1];
[1]*NovImmune SA, Geneva, Switzerland,* [2]*INSERM, Lyon, France,* [3]*Flow cytometry core facility, Geneva, Switzerland.*

Introduction: A CD47xCD19 bispecific antibody (BsAb) is being developed for the treatment of B cell malignancies. We investigated if B cell receptor (BCR) signalling, a key pathway targeted in auto-immune diseases and B cell malignancies, is altered in the presence of the BsAb. Materials and Methods: Using flow cytometry-based assays, we investigated whether co-ligation of CD19xCD47 could inhibit BCR-crosslinking-induced B cell proliferation and alter cell surface dynamics of BCR/CD19. Western blot and gene array were used to investigate the downstream signaling pathways. Results: We confirmed the role of CD19 in regulating BCR-signaling by showing that bivalent targeting of CD19 with a monoclonal antibody (mAb) inhibited BCR-induced cell proliferation. In contrast, monovalent engagement of CD19 failed to abrogate B cell proliferation. Interestingly, the CD47xCD19 BsAb inhibited B cell proliferation more potently than the CD19 mAb. The inhibitory effect of the BsAb was not attributable to CD47 binding alone as the anti-CD47 mAb had no effect on B cell proliferation. Co-engaging CD19xCD47 prevented CD19 clustering and its migration to the BCR. The CD19 mAb showed no impact on either CD19 clustering or its migration to the BCR. The reduced CD19 migration to the BCR resulted in a decreased CD19 phosphorylation upon BCR cross-linking. Gene array analysis confirmed that the BsAb and the anti-CD19 mAb employed different mechanisms in controlling B cell activation. Conclusions: These data suggest that the BsAb may provide therapeutic benefit in settings of autoimmunity and B cell malignancies by dampening BCR-stimulated B cell proliferation.

WS.C3.01 Transplantation - pathogenesis and early diagnosis

WS.C3.01.01
Improved risk stratification of pretransplant donor-specific antibodies with epitope analyses

***E. G. Kamburova**, on behalf of the PROCARE consortium, H. G. Otten;*
University Medical Center Utrecht, Utrecht, Netherlands.

The presence of pretransplant donor-specific anti-HLA antibodies (DSA) is associated with increased risk of kidney graft failure. HLA antibody detection by single-antigen bead assay (SAB) is much more sensitive than by complement-dependent crossmatches (CDC-XM). Here, we studied the impact of SAB-detected DSA on graft survival for all CDC-XM negative kidney transplants performed between 1995 and 2006 in the Netherlands. The impact was most pronounced in the 3237 deceased-donor transplantations: transplantations positive for SAB detected DSA (N=430/3237) had a 16% worse 10-year graft survival than those without DSA. Due to the lack of second-field donor HLA typing, donor-specificity of the SAB-detected antibodies was initially determined at serological (split) level. The SAB assay however allows for second-field definition of antibody-specificity. Using the HLA-epitope registry (http://www.epregistry.com.br) the most likely epitope-specificity of the detected antibodies was defined. NMDP-HLA-haplotype frequencies were used to determine the most likely second-field HLA types of all recipients and donors.
Combination of these tools enabled the determination of donor anti-HLA-epitope specific antibodies (DESA). Pretransplant DESA-positive deceased-donor transplantations (N=312/3237) had a 20% poorer 10-year graft survival than those without DESA. A higher number of DESA led to an even worse graft survival: transplantations with more than 2 DESA (N=236) had a 25% poorer graft survival compared to transplantations without DESA. We conclude that even without the exact knowledge of both the HLA-epitope specificity of the SAB-detected antibodies and the mismatched donor HLA-epitopes, the number of pretransplant DESA might be a better parameter to stratify risk than the presence of serologically-defined DSA.

WS.C3.01.02
Detection of alloantibodies against NK cell antigens after transplantation

***T. Langer Jacobus**, R. E. Schmidt, C. S. Falk, E. Jäckel, F. W. Vondran, C. Ferreira de Figueiredo, R. Jacobs;*
MHH, Hannover, Germany.

Introduction: Chronic antibody-mediated rejection (ABMR) is known to play a key role in graft survival, dysfunction or rejection and is still a major obstacle in transplantation success. Recent studies have shown the importance of non-HLA antigens in the formation of donor-specific antibodies (DSA) in graft rejection. Initial experiments revealed the presence of allospecific antibodies against antigens encoded in the donor NK cell gene complex (NKC) and leukocyte receptor gene complex (LRC) in 23% of the sera of liver and kidney recipients (n=92). This prompted us to further investigate the frequency and relevance of alloantibody formation against donor NK cell receptors in graft recipients after solid organ transplantation.
Materials and Methods: Recombinant proteins for the predicted most relevant antigens were produced and coupled to differentially coloured-multiplex beads which were used to screen patient sera for the presence of antibodies on a FACSCanto II flow cytometer.
Results: This screening strategy revealed antigen-allospecific antibodies against various NKC- and LRC-encoded receptors such as KIR2DL2, KIR2DS1, LILRB3 in 5 to 17% of liver, lung and kidney recipients.
Conclusions: The data indicate a high degree of potential mismatch in NK cell diversity between donor and recipient in the case of solid organ transplantation. Further analyses will be performed in order to evaluate the functional consequences and clinical relevance of these antibodies in transplantation.
Supported by DFG grant SFB738/A5
1

WS.C3.01.03
HLA Class I Haplotype Diversity Is Consistent with Selection for Frequent Existing Haplotypes

***Y. Louzoun**[1], I. Alter[1], M. Maiers[2], L. Gragert[3];*
[1]Bar Ilan, Ramat Gan, Israel, [2]CIMBTR, Minneapolis, United States, [3]Tulane University, New Orleans, United States.

The major histocompatibility complex (MHC) contains the most polymorphic genetic system in humans, the human leukocyte antigen (HLA) genes of the adaptive immune system. High allelic diversity in HLA is argued to be maintained by balancing selection, such as negative frequency-dependent selection or heterozygote advantage. Selective pressure against immune escape by pathogens can maintain appreciable frequencies of many different HLA alleles.
The selection pressures operating on combinations of HLA alleles across loci, or haplotypes, have not been extensively evaluated since the high HLA polymorphism necessitates very large sample sizes, which have not been available until recently. We aimed to evaluate the effect of selection operating at the HLA haplotype level by analyzing 5 locus haplotype frequencies derived from over six million individuals genotyped by the NMDP registry.
In contrast with alleles, HLA haplotype diversity patterns suggest purifying selection, as certain HLA allele combinations co-occur in high linkage disequilibrium. Linkage disequilibrium is positive (D'>0) among frequent haplotypes and negative (D'<0) among rare haplotypes. Fitting the haplotype frequency distribution to several population dynamics models, we found that the best fit was obtained when significant positive frequency-dependent selection (FDS) was incorporated. Finally, the Ewens-Watterson test of homozygosity showed excess homozygosity for 5-locus haplotypes within 23 US populations studied.
Haplotype diversity is most consistent with purifying selection for HLA Class I, and was not inferred for HLA Class II. We discuss our empirical results in the context of evolutionary theory, exploring potential mechanisms of selection that maintain high linkage disequilibrium in MHC haplotype blocks.

WS.C3.01.04
High numbers of donor-specific IL-21 producing cells predict rejection after kidney transplantation

***N. M. van Besouw**[1], L. Yan[1,2], R. de Kuiper[1], M. Klepper[1], D. Reijerkerk[1], D. L. Roelen[3], F. H. Claas[3], M. C. Clahsen-van Groningen[1], D. A. Hesselink[1], C. C. Baan[1];*
[1]Erasmus MC, Rotterdam, Netherlands, [2]Trans West China Hospital, Chengdu, China, [3]LUMC, Leiden, Netherlands.

Introduction: Both IFN-γ and IL-21 support induction and expansion of highly-reactive cytotoxic $CD8^+$ T-cells. In addition, IL-21 is a key cytokine for differentiation of alloantigen activated naïve and memory B-cells into antibody-producing plasma cells. We questioned whether the donor-specific IFN-γ and IL-21 producing cells (pc) frequency can predict kidney transplant rejection.
Methods: PBMC samples from 47 patients obtained at 6 months after kidney transplantation of whom 14 patients developed a late rejection (>6 months). In addition, pre-transplantation samples of 38 patients of whom 17 patients had an early rejection (<3 months). The frequency of donor-reactive circulating IFN-γ and IL-21 pc was determined by Elispot assay.
Results: Remarkably, no relation was found between donor-specific IFN-γ pc frequency and rejection in both groups. However, significantly higher donor-specific IL-21 pc numbers were found in patients who developed rejection compared to those without rejection in both the late (p=0.020) and early (p=0.024) rejection group. ROC-curve analysis of donor-specific IL-21 pc frequencies distinguished the development of rejection from non-rejection with a specificity of 88% and 80% in the late and early rejection group, and a sensitivity of 50% and 73%, respectively. Patients with low IL-21 pc frequencies had a significantly increased rejection free survival rate in both the late (p=0.0008) and early rejection group (p=0.0005) compared to those with high frequencies.
Conclusion: The frequency of donor-specific IL-21 producing cells is linked to an increased risk of rejection, giving it the potential to be a new biomarker in predicting rejection in different phases of transplantation.

WS.C3.01.05

Immunization against the polysaccharide Poly-N-acetylglucosamine reduces graft-versus-host disease without affecting microbial diversity

J. Hülsdünker[1,2,3], *O. S. Thomas*[1,2,3], *Z. Gao*[4], *S. Duquesne*[1], *S. Kirschnek*[5], *A. Schmitt-Graeff*[6], *M. J. Blaser*[4], *G. B. Pier*[7], *R. Zeiser*[1];
[1]*Department of Hematology, Oncology and Stem Cell Transplantation, Freiburg, Germany,* [2]*Spemann Graduate School of Biology and Medicine (SGBM), University Freiburg, Germany,* [3]*Faculty of Biology, University Freiburg, Germany,* [4]*Department of Medicine, New York University Langone Medical Center, New York, United States,* [5]*Institute of Medical Microbiology and Hygiene, University Medical Center Freiburg, Freiburg, Germany,* [6]*Institute of Surgical Pathology, Faculty of Medicine, Freiburg, Germany,* [7]*Division of Infectious Diseases, Department of Medicine, Brigham and Women's Hospital/Harvard Medical School, Boston, United States.*

Acute graft-versus-host disease (aGVHD) is a severe complication after allogeneic hematopoietic cell transplantation (allo-HCT). Opportunistic infections, especially during the period of neutropenia, are frequently occurring in allo-HCT patients and demand antibiotic intervention to prevent bacteremia and sepsis. However antibiotics lead to a loss of microfloral diversity which is connected to a higher incidence of aGVHD. Antibacterial therapies that eliminate invading bacteria without impacting the diversity of the microflora are therefore highly desirable. A potential solution offer anti-bacterial antibodies that target pathogens ultimately leading to elimination by innate immune cells.
We investigated the potency of active and passive immunization against the polysaccharide Poly-N-acetylglucosamine (PNAG) that is expressed on numerous pathogens in a mouse model of aGVHD. Anti-PNAG antibody treatment in the early phase after allo-HCT reduced aGVHD-related mortality and histopathological aGVHD severity. The treatment did not disturb the intestinal microbial composition as determined by 16S rDNA sequencing. Mechanistically we could show that neutrophils were recruited more abundantly to the intestinal tract following anti-PNAG treatment but exhibited a reduced activation profile as determined by their myeloperoxidase activity. Vaccination against PNAG prior to allo-HCT induced high antibody titers in mice that were detectable throughout the early phase of GVHD and significantly reduced GVHD-related mortality.
In summary, antibody-treatment-based and vaccination-based anti-PNAG immunization strategies open a new strategy to interfere with aGVHD without affecting microbial diversity.

WS.C3.01.06

Motifs of peptides binding in HLA-DP and their relation with T-cell epitope groups relevant in stem cell transplantation

P. Van Balen[1], *M. G. Kester*[1], *W. De Klerk*[1], *P. Crivello*[2], *A. H. De Ru*[1], *M. Van de Meent*[1], *I. Jedema*[1], *M. H. Heemskerk*[1], *K. Fleischhauer*[2], *J. H. Falkenburg*[1], *P. A. Van Veelen*[1];
[1]*Leiden University Medical Center, Leiden, Netherlands,* [2]*Institute for Experimental Cellular Therapy, Essen, Germany.*

Introduction Permissiveness of HLA-DP mismatches in allogeneic stem cell transplantations is based on categorisation of HLA-DP alleles into T-cell epitope (TCE) groups. TCE1 and TCE2 are clearly defined, but TCE3 represents a relatively heterogeneous group. To investigate whether characterization of peptides binding in HLA-DP can be of use to re-define TCE groups, we analyzed the peptidome of 11 HLA-DP molecules. **Methods** To investigate peptides presented in HLA-DP encoded by DPB1*09:01, 10:01, 17:01 (TCE1), DPB1*03:01, 14:01 (TCE2) and DPB1*01:01, 02:01, 04:01, 04:02, 05:01, 13:01 (TCE3), HLA-DPB1 typed EBV-LCL were expanded. HLA-DP immunoaffinity chromatography using anti-HLA-DP B7.21 antibody was performed, followed by analysis of eluted peptides using mass spectrometry and subsequent alignment and clustering using Gibbs sampling to obtain motifs of peptides binding in different HLA-DP molecules.
Results All alleles from TCE1 had a similar KAL motif at P1, P6 and P9, reflecting their structural similarity in the relevant hypervariable regions. This motif was shared also by DPB1*14:01 from TCE2, while the motif for HLA-DPB1*03:01 was different (RAS). TCE3 alleles could be classified into two main groups: those with a totally different FFV motif at P1, P6 and P9 (DPB1*02:01, 04:01, 04:02 with high structural similarity), and those with a motif more similar to TCE1 in P1, but different in P6 and P9 (KXX), (DPB1*01:01, 05:01 and 13:01). **Conclusion** The current categorization into TCE groups may need to be adjusted based on these results, especially with regard to potentially permissive or non-permissive mismatches within HLA-DP alleles in TCE group 3.

WS.C3.02 T regulatory cells derived and other cellular therapies in transplantation

WS.C3.02.01

Development of a novel protocol to produce massive amounts of autologous thymus-derived Treg cells (thyTreg) to be employed as cellular therapy in transplanted children. A new paradigm in the treatment of immunological diseases

E. Bernaldo de Quirós[1], *V. Pérez*[1], *M. Clemente*[2], *J. Gil-Jaurena*[3], *M. Fernández-Santos*[4], *S. Suárez*[4], *V. Plasencia*[4], *A. Acosta*[4], *M. Camino*[5], *N. Gil*[5], *E. Panadero*[5], *C. Medrano*[5], *M. Pion*[1], *R. Correa-Rocha*[1];
[1]*Laboratory of Immune-regulation, Gregorio Marañón Health Research Institute, Madrid, Spain,* [2]*Cell Culture Unit, Gregorio Marañón Health Research Institute, Madrid, Spain,* [3]*Pediatric Cardiac Surgery Unit, Gregorio Marañón Hospital, Madrid, Spain,* [4]*Good Manufacturing Practice (GMP) Facility, Gregorio Marañón Health Research Institute, Madrid, Spain,* [5]*Pediatric Cardiology Unit, Gregorio Marañón Hospital, Madrid, Spain.*

Introduction: Immune allograft rejection remains the main obstacle to successful transplants. Although transfer of regulatory T cells (Treg) has acquired growing interest in achieving indefinite graft survival, Treg isolated from peripheral blood showed several limitations. Therefore, we explored the use of thymic tissue as an alternative source of Treg for use as cellular immunotherapy.
Materials and methods: We have developed a novel GMP-compatible protocol to obtain Treg from thymuses routinely discarded during pediatric cardiac surgery. Quality and safety of the thyTreg product manufactured in the GMP facility have been confirmed. After receiving the approval from the Spanish Drug Agency (AEMPS), we will initiate the first clinical trial worldwide (phase I/IIa) to evaluate the safety and feasibility of employing autologous thyTreg in children undergoing heart transplantation.
Results: The thyTreg product obtained with our protocol showed very high purity (>90% of CD25+Foxp3+ cells), viability of >85% and very high suppressive capacity. Importantly, the number of thyTreg recovered from one thymus reached values of more than $13x10^9$ cells, enough to prepare more than 1500 doses of thyTreg. A single dose of autologous thyTreg will be infused back to the patient after transplantation and the other doses will be cryopreserved for potential future reinfusions.
Conclusions: Massive amounts of pure, highly suppressive Thy-Treg obtained with our novel GMP-compatible protocol are suitable for use as cellular immunotherapy to prevent rejection in heart-transplanted children. The clinical use of these thyTreg could increase the graft survival in transplanted patients, and may revolutionize the treatment of other immunological diseases.

WS.C3.02.02

Effective Treg therapy of lethal acute graft-versus-host disease

C. Riegel[1], *T. Böld*[1], *E. Huber*[2], *P. Hoffmann*[1,3], *M. Edinger*[1,3];
[1]*University Hospital Regensburg, Regensburg, Germany,* [2]*University Regensburg, Regensburg, Germany,* [3]*Regensburg Center for Interventional Immunology (RCI), University Regensburg, Regensburg, Germany.*

Allogeneic stem cell transplantation (SCT) is the treatment of choice for many hematologic diseases, carrying the inherent risk of acute graft-versus-host disease (aGVHD) that is caused by alloreactive donor T cells and mainly targets the gastrointestinal (GI) tract, skin and liver. We previously showed that co-transplantation of donor $CD4^+CD25^+$ regulatory T cells (Treg) prevents lethal aGVHD even in complete MHC-mismatched SCT models, a strategy already confirmed in first clinical trials. Here, we examined the efficacy of donor Treg to treat established aGVHD. We isolated and *in vitro* expanded $CD4^+CD25^+CD62L^+$ Treg for 14d from BALB/c donors that multiplied up to 80-fold and upregulated GI-tract homing receptors *in vitro*. Upon transfer into CB6F1 mice with established aGVHD (d11 post SCT) they rescued >65% of recipients from otherwise lethal aGVHD and still 30% if administered in very late disease stages (d32 post SCT). Initially, therapeutically administered Treg (taTreg) migrated predominantly into the gastrointestinal tract. Here, they reduced the influx of neutrophils and conventional T cells into the colon and diminished their proliferation and secretion of pro-inflammatory cytokines. taTreg persisted long-term, maintained their phenotype and Foxp3 expression in GVHD and non-GVHD target organs and still represented 5-25% of the local Treg population by d100 post SCT. They promoted tissue regeneration in the GI tract, as indicated by the reappearance of Paneth cells, and blocked further lymphoid tissue destruction thereby fostering immune reconstitution. Summarized, our results demonstrate that donor Treg transfer seems effective for the treatment of severe aGVHD. *Supported by DFG.*

WS.C3.02.03

Re-directing Treg specificity toward allograft transplants to sustainably prevent rejection

R. Alhamawi[1,2]*, C. Dempsey*[1]*, R. Albugami*[1]*, S. Lee*[1]*, N. D. Jones*[1]*;*
[1]*Institute of immunology and immunotherapy, Birmingham, United Kingdom,* [2]*Medical laboratory technology department, Madinah, Saudi Arabia.*

Foxp3$^+$ regulatory T cells (Tregs) are cells with a potent immunosuppressive capacity that may be used as a cellular therapy to suppress autoimmunity and transplant rejection. It has been suggested that redirecting the specificity of Tregs to alloantigen might increase their potency to suppress rejection compared to polyclonal cells. With this in mind, we have optimised a protocol to expand mouse Tregs *in vitro* using anti-CD3/CD28 beads in the presence IL-2 and TGFβ to facilitate genetic manipulation. This protocol allowed a significant 4-6 fold expansion of Tregs with limited contamination with conventional T cells. In an assay of non-specific suppression where both conventional T cells (Tcons) and Tregs were activated by anti-CD3/anti-CD28 mAbs, expanded Tregs were found to suppress the conventional T cell response at least as well as freshly isolated Tregs. Importantly, the expanded Tregs proved to be amenable to retroviral-transduction with a plasmid carrying T cell receptor (TCR) α and β chains that confer reactivity to the MHC class I alloantigen H2K^b. Analysis of transduced Tregs revealed that 85%±5% expressed the alloreactive TCR following transduction. Finally, the TCR gene-engineered Tregs were tested for their ability to suppress an allogeneic CD4$^+$ and CD8$^+$ T cell response *in vitro*. We found that the TCR engineered Tregs suppressed alloreactive T cell responses with increased potency compared to expanded mock-transduced Tregs *in vitro*. Taken together these data suggest that indeed re-directing Tregs to transplant antigens may improve the efficacy of these cells when used as a cellular therapy to prevent transplant rejection.

WS.C3.02.04

Extracellular adenosine reversibly inhibits the activation of human regulatory T cells and negatively influences the achievement of the operational tolerance in liver transplantation

A. Baroja-Mazo[1]*, B. Revilla-Nuin*[1]*, L. Martínez-Alarcón*[1]*, J. Herrero*[2]*, A. El-Tayeb*[3]*, C. Müller*[3]*, P. Aparicio*[4]*, P. Pelegrín*[1]*, J. Pons*[1]*;*
[1]*Biomedical Research Institute of Murcia (IMIB-Arrixaca), EL PALMAR- MURCIA, Spain,* [2]*Clínica Universidad de Navarra, Pamplona, Spain,* [3]*Pharma Center Bonn, Bonn, Germany,* [4]*University of Murcia, MURCIA, Spain.*

The possibility of stimulating or inducing a state of operational tolerance in transplantation is gaining strength. In murine models, a differential role of extracellular adenosine (eADO) for regulatory (Tregs) and effector (Teffs) T cells has been proposed: inhibiting Teffs activity and inducing Tregs. The aim of the present study was to analyze the action of extracellular nucleotides in human T cells, and moreover, examine the influence of CD39 and CD73 ectonucleotidases and subsequent adenosine signaling through adenosine 2 receptor (A_2R) in the induction of clinical tolerance after liver transplantation. The action of extracellular nucleotides in human T cells was analyzed by *in vitro* experiments with isolated T cells. Additionally, 17 liver transplant (LT) patients were enrolled in an immunosuppression withdrawal trial, and the differences in the CD39-CD73-A_2R axis were compared between tolerant and non-tolerant patients. In contrast to the murine model, the activation of human Tregs was inhibited in a similar rate to Teffs in the presence of eADO although this inhibition was reversible. Moreover, the relative expression of the enzyme responsible for the irreversible degradation of ADO, adenosine deaminase (*ADA*), was much higher in tolerant patients with respect to the non-tolerant group along the immunosuppression withdrawal process. Our data support the idea that extracellular ADO signaling and its degradation by ADA may play a role in the complex system of regulation of liver transplantation tolerance. In addition, we show the capacity of *ADA* expression to differentiate the operational tolerance state at the first steps of the immunosuppression withdrawal process.

WS.C3.02.05

Targeting recipient antigen presenting cells with sialic acid- modified alloantigen to promote transplantation tolerance

M. Sen[1]*, K. Ratnasothy*[2]*, M. Ambrosini*[3]*, D. Guiliano*[1]*, Y. van Kooyk*[3]*, G. Lombardi*[2]*, L. A. Smyth*[1,2]*;*
[1]*School of Health, Sport and Bioscience, University of East London, London, United Kingdom,* [2]*MRC Centre for Transplantation, King's College London, London, United Kingdom,* [3]*Department of Molecular Cell Biology and Immunology, VU University Medical Centre, Amsterdam, Netherlands.*

Sialic acid-binding immunoglobulin-like lectins (siglec) are inhibitory receptors expressed on dendritic cells (DCs) that bind to sialic acid ligands. These receptors have previously been targeted to murine DCs to induce antigen-specific tolerance in an autoimmune mouse model. To date, it has not been established whether targeting these receptors can induce transplantation tolerance; therefore our aim was to target siglec-expressing recipient DCs with sialic acid- modified donor alloantigen MHC class I K^d peptide (sia-alloantigen) to promote organ transplant survival.
Siglec expression on bone marrow-derived DCs (BMDCs) and binding of FITC- conjugated sia- alloantigen was confirmed by flow cytometry. Following sia- alloantigen treatment, targeted BMDCs had a reduced capacity to induce antigen specific CD4$^+$ T cell proliferation and effector cytokine production. In addition, co-culturing antigen specific CD4$^+$ T cells with sia- alloantigen treated BMDCs, led to a 3-fold percentage increase in CD4$^+$FOXP3$^+$ regulatory T cells (Tregs).
Flow cytometry analysis suggests that sia- alloantigen binds to DCs *in vitro* and *in vivo*. Targeting sia- alloantigen to DCs in B6.RAG-2$^{-/-}$ recipient mice reconstituted with T cells, led to significant skin graft prolongation. To assess which DC subset was involved, B6.BATF3 $^{-/-}$ mice, which lack the CD8α$^+$ and CD103$^+$ DC subsets were compared with B6 mice. Skin transplant prolongation, reduced alloantibody production and an increased proportion of Tregs were observed only in B6.BATF3 $^{-/-}$ mice after treatment with sia- alloantigen.
In conclusion, targeting siglecs on recipient CD8α negative DCs with sia- alloantigen may represent a novel DC targeting regimen to regulate allorecognition.

WS.C3.02.06

Third party virus-specific T-cells for treatment of viral reactivations in immune compromised patients and risks of allo-HLA cross-reactivity

W. Huisman[1,2]*, D. A. Leboux*[2]*, L. Hageman*[2]*, D. Amsen*[1]*, F. J. Falkenburg*[2]*, I. Jedema*[2]*;*
[1]*SANQUIN, Amsterdam, Netherlands,* [2]*LUMC, Leiden, Netherlands.*

Adoptive transfer of partially HLA-matched virus-specific T-cells from healthy third party donors is a potential strategy to temporarily provide anti-viral immunity to immune-compromised patients. However, these T-cells harbor the risk of mediating allo-HLA cross-reactivity. To assess the magnitude of this risk, we examined the occurrence and diversity of allo-HLA cross-reactivity mediated by T-cell populations targeting different proteins from cytomegalovirus (CMV), Epstein-Bar virus (EBV) and adeno virus (AdV) using an allogeneic EBV-LCL panel covering 116 different HLA molecules and secretion of IFNγ as read-out. Allo-HLA cross-reactivity patterns were confirmed using K562 cells expressing single HLA molecules upon retroviral transduction. A significant proportion of HLA-A*01/A*02/B*07/B*08-restricted virus-specific T-cell populations (n=170) isolated from 27 healthy donors (30% of the CMV, 46% of the EBV and 36% of the AdV-specific T-cell populations tested) exerted allo-HLA cross-reactivity against one or more allogeneic EBV-LCLs in this analysis. However, for some specificities (e.g. HLA-A*02:01-restricted EBV-LMP2-FLY) the allo-HLA cross-reactivity was limited (n=1/11), whereas for other specificities (e.g. HLA-B*08:01-restricted EBV-BZLF1-RAK) almost all tested populations (n=9/13) showed profound allo-HLA cross-reactivity. Separate analysis of subcultures, sorted on TCR-Vβ usage from the bulk single specificity T-cell population, revealed several specific allo-HLA cross-reactivity patterns. Furthermore, recurrent patterns of allo-HLA cross-reactivity were observed for virus-specific T-cell cultures expressing the same TCR-Vβ family that were isolated from different donors. These data indicate that allo-HLA cross-reactivity by third party virus-specific T-cells can be a serious problem and that the magnitude and diversity may be associated with the level of T-cell receptor oligoclonality within a certain T-cell population.

WS.C4.01 Manipulation of tolerogenic pathways

WS.C4.01.01

PD-1 regulates the expression of HELIOS in self-reactive CD8$^+$ and CD4$^-$CD8$^-$T cells

N. Rodriguez Rodriguez[1,2]*, S. A. Apostolidis*[3]*, E. Montes Servin*[4]*, O. Arrieta*[4]*, G. C. Tsokos*[3]*, F. Rosetti*[1]*, J. C. Crispin*[1]*;*
[1]*Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Mexico,* [2]*Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico,* [3]*Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, United States,* [4]*Instituto Nacional de Cancerologia, Mexico City, Mexico.*

Self-reactive CD8$^+$ lymphocytes that recognize cognate antigen in peripheral tissues become inactivated, downregulate CD8, and express PD-1, becoming PD-1$^+$ double negative (DN, CD4$^-$CD8$^-$TCR-αβ$^+$) T cells, a population of lymphocytes increased in autoimmunity. However, whether PD-1 plays a role in DN T cells is unknown.
Transfer of PD-1-deficient OT-I CD8$^+$ T cells into mice that express ovalbumin as self-antigen resulted in an increased expansion of DN T cells with an elevated production of pro-inflammatory cytokines (e.g. IFN-γ), a result that was also observed under steady state conditions in *Pdcd1*-deficient mice.
Further analyses revealed that HELIOS (*Ikzf2*), a transcription factor important for immune tolerance, was specifically expressed by DN cells upon self-antigen encounter. Absence of PD-1 or PD-L1, as well as blockade of PD-1 during early self-antigen encounter, abrogated the upregulation of HELIOS, suggesting that signaling via PD-1 is necessary for HELIOS induction *in vivo*. *In vitro* experiments confirmed that presence of PD-L1 during T cell activation enabled a stable induction of HELIOS, and demonstrated that PD-1-mediated PI3K/AKT inhibition promotes its expression.
In patients with non-small cell lung carcinoma that received pembrolizumab, anti-PD-1 administration significantly reduced the frequency of HELIOS$^+$ DN T cells together with a reciprocal elevation of Ki-67$^+$ DN T cells.
RNA-sequence and shRNA studies proved that HELIOS is a central hub that regulates several transcriptional effects downstream of PD-1 signaling required to curb T cell activation.
In conclusion, by promoting HELIOS expression, which regulates the PD-1-imposed transcriptional program, PD-1 restrains the expansion of self-reactive CD8 and DN lymphocytes.

WS.C4.01.02

Immunotherapy with apitopes® blocks the immune response to thyroid stimulating hormone receptor in HLA-DR transgenic mice

E. Schurgers[1], *F. La Greca*[1], *D. C. Wraith*[1], *K. F. Martin*[2], *L. Jansson*[1];
[1]*Apitope International NV, Diepenbeek, Belgium,* [2]*Apitope Technology (Bristol) ltd, Chepstow, United Kingdom.*

Graves' disease is an endocrine, autoimmune disorder mediated by autoreactive T and B lymphocytes responding to the thyroid-stimulating hormone receptor (TSHR). Stimulatory anti-TSHR antibodies activate thyroid cells, resulting in typical Graves' hyperthyroidism. Although current treatment options are effective in initially eliminating the hyperthyroidism, they are not able to interrupt the autoimmune processes in Graves' disease. Long-term eradication of hyperthyroidism in Graves' disease patients could be achieved by antigen-specific immunotherapy as the formation of anti-TSHR antibodies is T cell dependent.
We designed a peptide-based antigen-specific immunotherapy to specifically re-establish immune tolerance to TSHR through the development of antigen-processing-independent epitopes (apitopes®). Combining MHC binding assays with immunization and tolerance induction experiments in human leukocyte antigen HLA-DRB1*0301 transgenic (DR3tg) mice, TSHR immune dominant peptides were identified. The combination of these TSHR-derived peptides induced T cell tolerance towards TSHR in DR3tg mice. In addition, a challenge model was created in DR3tg mice using an adenovirus expressing the extracellular domain of TSHR. In this animal model, a mixture of two immunodominant apitopes® was sufficient to suppress the anti-TSHR antibody production by more than 90%. Thus, selected peptides efficiently regulate the anti-TSHR T and B cell responses, specifically the generation of anti-TSHR antibodies, in DR3tg mice. Furthermore, selected peptides were assessed for their antigenicity using PBMC samples from Graves' disease patients, demonstrating the identification of relevant human T cell epitopes. These results demonstrate that antigen-specific immunotherapy with apitopes® from TSHR is a suitable approach, and is currently undergoing clinical trials, for the treatment of Graves' disease.

WS.C4.01.03

Pathogenic antibody development and blister formation due to impaired peripheral tolerance

S. Haeberle[1], *X. Wei*[1], *K. Bieber*[2], *S. Goletz*[2], *E. Schmidt*[2], *R. Ludwig*[2], *A. Enk*[1], *E. Hadaschik*[3,1];
[1]*Department of Dermatology, Heidelberg, Germany,* [2]*Department of Dermatology, Lübeck, Germany,* [3]*Department of Dermatology, Essen, Germany.*

Missing functional regulatory T-cells (Treg) lead to development of severe autoimmune inflammation. Scurfy mice have a mutation in foxp3 and thereby no functional Tregs. Scurfies show severe skin inflammation with Th1/Th2 prone immune response and high titers of autoantibodies against skin proteins. We wanted to analyze if autoimmune blistering diseases (AIBD) develop in the absence of Treg. We screened scurfy sera and found elevated levels of autoantibodies against different AIBD-related autoantigens. Histological examination of scurfy skin, showed the presence of subepidermal blisters. Together with deposition of autoantibodies in the skin, we demonstrate that scurfies develop the phenotype of AIBD. We generated hybridomas, to isolate monoclonal autoantibodies (mAb) from scurfies. Mass spectrometry analysis revealed BP230 as the autoantigen of one of these mAb (20B12). We confirmed the antigen by immunofluorescence, westernblot and ELISA. Furthermore, we proved pathogenicity by injection of 20B12 into neonatal mice which led to subepidermal blister formation in 70% of mice. To investigate the interaction between autoreactive CD4+ T-cells from Scurfies and B-cells, CD4+ T-cells from scurfy and WT mice were transferred into *nu/nu* mice. Transfer of autoreactive scurfy CD4+ T-cells into *nu/nu* mice, resulted in autoantibody production and subepidermal blister formation similar to scurfy. In summary, we show that impaired peripheral tolerance leads to an AIBD phenotype with pathogenic autoantibody production and blister formation in scurfy mice. Furthermore the injection of anti-BP230 autoantibody was sufficient to induce blister formation, therefore BP230 should be considered as a relevant target autoantigen in AIBD. Funded by SFB/TR156.

WS.C4.01.04

APRIL induces a novel subset of IgA$^+$ regulatory B cells that suppress inflammation through the expression of IL-10 and PD-L1

C. M. Fehres[1,2], *N. O. Van Uden*[1,2], *N. G. Yeremenko*[1,2], *L. Fernandez*[3], *G. Franco Salinas*[1], *L. M. Van Duivenvoorde*[1,2], *B. Huard*[4], *J. Morel*[5], *H. Spits*[2], *M. Hahne*[3], *D. L. Baeten*[1,2];
[1]*Department of Clinical Immunology and Rheumatology, Academic Medical Center, Amsterdam, Netherlands,* [2]*Department of Experimental Immunology, Academic Medical Center, Amsterdam, Netherlands,* [3]*Institut de Genetique Moleculaire de Montpellier, Centre National de la Recherche Scientifique, Universite de Montpellier, Montpellier, France,* [4]*Institute for Advanced Biosciences , University Grenoble Alpes , INSERM U1209, Grenoble, France,* [5]*Department of Rheumatology, CHU de Montpellier, Montpellier University, Montpellier, France.*

Regulatory B cells (Bregs) are immunosuppressive cells that modulate immune responses through multiple mechanisms, such as the production of IL-10 and the skewing of T cell differentiation in favor of a regulatory phenotype. However, the signals required for the differentiation and activation of these cells remain still poorly understood. As we have previously found that APRIL promoted IL-10 production in human B cells, we hypothesized that APRIL, but not BAFF, may be involved in the induction and/or activation of IL-10 producing Bregs that suppress inflammatory responses *in vitro* and *in vivo*. CD19$^+$IgD$^+$CD27$^-$ naïve B cells were cultured in the presence of IL-21+TGF-β, IL-21+APRIL or IL-21+BAFF to induce class switch recombination to IgA.
Regulatory B cell functions and phenotypes were assessed on the class switched IgA B cells. Here, we describe that APRIL promotes the differentiation of naïve human B cells to IL-10-producing IgA$^+$ B cells. These APRIL-induced IgA$^+$ B cells display a Breg phenotype and inhibit T cell and macrophage responses *in vitro* through expression of IL-10 and PD-L1. Moreover, APRIL-induced IL-10 producing Bregs suppress inflammation *in vivo* in experimental autoimmune encephalitis (EAE) and contact hypersensitivity (CHS) models. Finally, we showed a strong correlation between APRIL and IL-10 in the inflamed synovial tissue of inflammatory arthritis patients. In conclusion, we have identified a novel subset of regulatory B cells within the IgA$^+$ B cell population that suppresses inflammation *in vitro* and *in vivo*, which indicate the potential relevance of this subset of B cells for immune homeostasis and immunopathology.

WS.C4.01.05

Molecular Mechanisms of T cell Tolerance Induction

S. T. H. Ng, *S. Bevington, P. Cockerill, D. C. Wraith;*
University of Birmingham, Birmingham, United Kingdom.

Escalating peptide immunotherapy is effective in treating experimental autoimmune encephalomyelitis (EAE) in mice and multiple sclerosis in humans. EAE can be prevented and treated by subcutaneous administration of the peptide MBP Ac1-9[4Y] in H-2^u mice. This protection from disease is dependent on IL-10 secreting Foxp3- cells. This project aims to look at the transcription, epigenetic and signalling profiles of CD4+ T cells as a result of peptide immunotherapy. Tg4 mice were treated with either peptide or vehicle control. mRNA-seq, DNAse I-seq and phospho-protein array analysis were carried out on CD4+ T cells isolated from the spleen. The results show that peptide immunotherapy induces Tr1-like cells with a tolerogenic transcription profile. DNAse I-seq results show an epigenetic landscape that is permissive to the expression of tolerogenic genes such as IL10. On the other hand, the down-regulation of inflammatory genes such as IL2 and TNFa is not explained by epigenetic changes. The phosphorylation status of downstream proteins of the TCR suggests that TCR signalling is impeded in Tr1-like cells explaining the limited expression of inflammatory genes.The data indicate that both epigenetic programming and modifications in signalling are important in generating Tr1-like cells. As peptide immunotherapy has been shown to be clinically relevant, it would be imperative to look for drug targets to further improve the efficacy and longevity of the treatment.

WS.C4.01.06

Macrophages treated with exosome-delivered miRNA-150 release immune suppressive exosome-like nanovesicles bearing antigen/MHC complex

K. Nazimek[1,2], *B. Nowak*[1], *M. Wasik*[1], *W. Ptak*[1], *P. W. Askenase*[2], *K. Bryniarski*[1,2];
[1]*Department of Immunology, Jagiellonian University Medical College, Krakow, Poland,* [2]*Section of Allergy and Clinical Immunology, Yale University School of Medicine, New Haven, United States.*

Introduction. T cell-derived, miRNA-150-carrying exosomes suppress mouse contact (CHS) and delayed-type (DTH) hypersensitivity reactions [J Allergy Clin Immunol 2013;132:170-81]. miRNA-150-carrying exosomes were shown to target antigen-presenting macrophages finally suppressing CHS effector cells [Immunology 2015;146:23-32]. Current studies aimed at determining the mechanism of immune suppression mediated by macrophages treated with exosome-miRNA-150.
Methodology. Wild type, OT-II or miRNA-150 KO mouse peritoneal macrophages were treated with T cell-exosomes and standardly cultured for 48 hours in serum-free medium. Resulting supernatant was filtered down to 0.22micrometers and ultracentrifuged at 100.000g for 70 minutes. Pelleted nanovesicles were used for treatment of adoptively transferred DTH effector T cells or analyzed cytometrically. OT-II macrophage-derived exosome-like nanovesicles were pre-incubated with anti-ovalbumin-323 antibodies. Ovalbumin-induced DTH effector T cells of OT-II mice were pre-incubated with ovalbumin-323 peptide.
Results. MHC class II-positive exosome-nanovesicles from culture of miRNA-150-pulsed macrophages of wild type, OT-II or miRNA-150 KO mice suppressed adoptively transferred effector T cells, and this inhibitory effect was enhanced by pre-incubation of pelleted nanovesicles with ovalbumin-323-specific antibodies and blocked by pre-incubation of effector T cells with ovalbumin-323 peptide.
Conclusions. Macrophages release suppressive exosome-like nanovesicles only after treatment with T cell-exosome-carried miRNA-150. As miRNA-150-pulsed macrophage-derived exosome-like nanovesicles express MHC class II and bind antigen-specific antibodies, we concluded that they display antigen/MHC complex. These exosome-like nanovesicles finally target effector T cells to suppress DTH immune response and this effect depends on the interaction of antigen/MHC complex expressed on macrophage-derived exosome-like nanovesicles with antigen-specific TCR of effector T cells.

WS.C4.02 Manipulation of Tolerance by FoxP3+T Regs

WS.C4.02.01

IL-21 sustains asthmatic and colitogenic CD4$^+$ T cell effector responses by promoting apoptosis of FoxP3$^+$ regulatory T cells

***L. Tortola**, H. Pawelski, S. S. Sonar, M. Kopf;*
ETH Zurich - Institute of Molecular Health Sciences, Zurich, Switzerland.

IL-21 is a key player of adaptive immunity with well-established roles in B cell- and cytotoxic T cell responses. IL-21 has also been implicated in the generation of effector CD4$^+$ T cells such as Th17 cells and inhibition of regulatory T cells (Tregs), but the mechanism and functional relevance of these findings remain controversial. We show here that IL-21 production by conventional Th2 cells drives allergic airway inflammation by intrinsic inhibition of FoxP3$^+$ Treg expansion. Mice lacking IL-21R displayed profoundly reduced generation of Th2 cells along with increased Tregs. In mixed bone marrow chimeras, we demonstrate that IL-21 promotes Th2 differentiation indirectly via inhibition of Tregs. Depleting the inflated Treg population in *Il21r*$^{-/-}$ mice restored Th2 generation and lung eosinophilia. Furthermore, IL-21 inhibited Treg generation in a model of Th1/17-driven colitis. Using elaborate competitive transfers of *Il21r*$^{+/+}$ and *Il21r*$^{-/-}$ CD4$^+$ cells into lymphopenic hosts, we show that IL-21 directly inhibited expansion rather than differentiation of Tregs, but was dispensable for development of Th1/Th17 effectors. Notably, we show that IL-21 sensitizes activated Tregs to caspase-dependent apoptosis by interfering with the expression of Bcl-2 family genes.
Collectively, our data demonstrates that IL-21 promotes apoptotic death of FoxP3$^+$ Treg cell intrinsically and thereby indirectly sustains generation of Th1, Th17 and Th2 cells and related effector responses, with profound implications in inflammatory pathologies. Blockade of IL-21 or IL-21R may be considered for regulatory T cell-based therapies of autoimmunity and allergy.

WS.C4.02.02

IL-10 signaling in CD11c$^+$ myeloid cells prevents gluten-dependent intraepithelial CD4$^+$ cytotoxic T lymphocyte infiltration and epithelial damage in the small intestine

***L. M. M. Costes**[1], D. J. Lindenbergh-Kortleve[1], L. A. van Berkel[1], S. Veenbergen[1], N. Papazian[1], Y. Simons-Oosterhuis[1], J. J. Karrich[2], R. M. Hoogenboezem[2], B. E. Clausen[3], T. Cupedo[2], J. N. Samsom[1];*
[1]Laboratory of Pediatrics, div. Gastroenterology and Nutrition, Erasmus Medical Center, Rotterdam, Netherlands, [2]Department of Hematology, Erasmus Medical Center, Rotterdam, Netherlands, [3]Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany.

Celiac disease (CeD) is a chronic small intestinal inflammatory disease occurring consequently to a breach of tolerance to gluten. In mice, IL-10 producing Tr1-like cells maintain gluten tolerance. Moreover, IL-10 preserves intestinal homeostasis by regulating CD11c$^+$ myeloid cells. Therefore, we hypothesized that disrupting IL-10 regulation of CD11c$^+$ myeloid cells would trigger a small intestinal inflammatory response to gluten.
Indeed, when reared on a gluten-containing diet, *Itgax*^{cre}*Il10ra*$^{fl/fl}$ mice lacking IL-10 receptor alpha on CD11c$^+$ myeloid cells developed CeD-like small intestinal inflammation. In particular, *Itgax*^{cre}*Il10ra*$^{fl/fl}$ mice displayed increased frequencies of small intestinal cytotoxic CD4^{pos}CD8αα^{pos} IEL (CD4 CTL) expressing high *Ifng*, a key player in CeD. CD4 CTL were negative for Ki67, suggesting that conversion of CD4$^+$ T-cells into CD4 CTL rather than expansion of an existing CD4 CTL pool caused increased frequency. Crucially, this was associated with epithelial stress involving increased epithelial MHCII expression and crypt hyperplasia and was gluten-dependent as both CD4 CTL frequency increase and epithelial stress were abolished in *Itgax*^{cre}*Il10ra*$^{fl/fl}$ mice reared on a gluten free diet. Small intestinal CD103$^+$CD11b$^-$ and CD103$^-$CD11b$^+$ dendritic cells and F4/80$^+$ macrophages preferentially took up orally-administered fluorescently-labeled gluten and had the highest number of significantly changed genes in an RNA sequencing analysis comparing small intestinal myeloid subsets of *Itgax*^{cre}*Il10ra*$^{fl/fl}$ mice and littermates. This suggests that IL-10 regulation of these myeloid populations prevents gluten-dependent conversion of small intestinal CD4 CTL and associated epithelial stress.
Altogether, our data indicate that disrupting IL-10 regulation of CD11c$^+$ myeloid cells causes gluten-dependent small intestinal inflammation with CeD features .

WS.C4.02.03

An autocrine TNFa - TNFR2 loop promotes epigenetic effects inducing human Treg stability *in vitro*

*P. C. Urbano, H. J. Koenen, I. Joosten, **X. He**;*
Radboudumc, Nijmegen, Netherlands.

A crucial issue for Treg-based immunotherapy is to maintain a bona fide Treg phenotype as well as suppressive function during and after *ex vivo* expansion. Several strategies have been applied to harness Treg lineage stability, including the addition of the mTOR inhibitor rapamycin to Treg cultures and the use of CD28-superagonist mAb. A TNFR2 agonist antibody was recently shown to favor homogenous expansion of Treg *in vitro*. Combined stimulation with rapamycin and TNFR2-agonist mAb enhanced hypo-methylation of the *FOXP3* gene, and thus promoting Treg stability. To further explore the underlying mechanisms of rapamycin and TNFR2-agonist mediated Treg stability, we here stimulated FACS-sorted human Treg with a CD28-superagonist mAb in the presence of rapamycin and TNFR2-agonist mAb. Phenotypic analysis of stimulated Treg revealed an autocrine loop of TNFα-TNFR2 underlying the maintenance of Treg stability *in vitro*. Addition of rapamycin to CD28-superagonist stimulated Treg led to a high expression of TNFR2, the main TNFR expressed on Treg, and additional stimulation with a TNFR2-agonist enhanced the production of TNFα.
Moreover, our data showed that the expression of histone methyltransferase EZH2, a crucial epigenetic modulator for potent Treg suppressor function, was enhanced upon stimulation with CD28-superagonist. Interestingly, rapamycin seemed to downregulate CD28-superagonist induced EZH2 expression, which could be rescued by the additional addition of TNFR2-agonist antibody. This process appeared TNFα dependent manner, since depletion of TNFα using Etanercept inhibited EZH2 expression. To summarize, we propose that an autocrine TNFα - TNFR2 loop plays an important role in endorsing Treg stability.

WS.C4.02.04

Interleukin-34 contributes to Tregs suppressive function and inhibits allogeneics responses

***A. FREUCHET**, S. BEZIE, C. USAL, V. DAGUIN, C. GUILLONNEAU, I. ANEGON;*
CRTI INSERM UMR 1064, Nantes, France.

Identify new specific and powerful mediators of immune tolerance is important. IL-34 is a cytokine that binds to CSF-1R, CD138 and PTPz and is involved in differentiation and survival of macrophages, as well as in suppression of certain immune responses. We showed that IL-34 is expressed by rodent and human Foxp3$^+$CD8$^+$/ CD4$^+$ Tregs. Additionally, the overexpression of IL-34 induces long-term allograft tolerance in a rat model through M2-macrophages differentiation as well as CD8$^+$/CD4$^+$ Tregs induction.
To further understand the function of IL-34 in immune responses, we generated IL-34-deficient rats thanks to CRISPR/Cas9 technology. These rats showed a decrease of CD8$^+$ and CD8$^+$CD45RC^{hi} T cells in the spleen and CD8$^+$ and CD8$^+$Foxp3$^+$ T cells in the blood compared to WT animals. The suppressive function of IL-34 deficient CD8$^+$CD45RC^{low} Tregs was not affected *in vitro* nor *in vivo* (wasting disease model in *Il2rg*$^{-/-}$ rats injected with T cells). However, IL-34-deficient CD4$^+$CD25$^+$CD127^{low} Tregs were not able to control the wasting disease, suggesting that IL-34 is essential for their suppressive function.
Finally, to analyze the potential of IL-34 in human allogeneics immune responses, we used a GVHD model in NSG mice injected with human PBMC. Treatment with IL-34 and rapamycin at suboptimal dose reduced the GVHD incidence vs. rapamycin or IL-34 alone (median survival: 39, 28 and 14 respectively; n=4-8). The role of Tregs in this model is under investigation.
Altogether, our data demonstrate the implication of IL-34 in CD4$^+$ Tregs function and the relevance of this cytokine as a regulatory cytokine in GVHD.

WS.C4.02.05

Repairing Foxp3 mutations in scurfy T cells restores regulatory T cell function

***l. jeker**, m. kornete;*
Department of Biomedicine and Transplantation Immunology & Nephrology, Basel, Switzerland.

Adoptive cell transfer is a powerful approach to treat various diseases including infectious diseases and certain blood cancers. Emerging genome engineering tools enable direct genetic manipulation of primary immune cells. This opens new therapeutic opportunities for monogenic T cell diseases. We recently reported an efficient protocol for CRISPR-mediated T cell editing. Here, we report the successful correction of two different pathogenic *Foxp3* mutations in primary murine T cells. Both repairing the cause of the scurfy syndrome, a 2bp insertion in *Foxp3,* and repairing the clinically relevant Foxp3^{K276X} mutation restored Foxp3 expression in primary T cells *in vitro*. Ex vivo gene-repaired T cells adoptively transferred to lymphodeficient mice survive and expand *in vivo*. We show that the gene-corrected cells are viable, express high levels of Foxp3, CD25 and other regulatory T cell (Treg) markers and are responsive to IL-2 treatment upon adoptive transfer *in vivo*. Importantly, repaired Treg cells prevent the development of dermatitis illustrating regained suppressive capacity *in vivo*. These results suggest that gene therapy of T cells might constitute an alternative to gene therapy in hematopoietic stem cells.

WS.C4.02.06

Epigenetic editing converts naive and memory CD4+ T cells into FOXP3 expressing regulatory T cells

C. Kressler[1], *D. Hamo*[1], *G. Gasparoni*[2], *J. Walter*[2], *A. Hamann*[1], *J. K. Polansky*[1];
[1]*German Rheumatism Research Center, Berlin, Germany,* [2]*Universität des Saarlandes, Saarbrücken, Germany.*

Regulatory T cells (Tregs) harbor a huge therapeutic potential as their immuno-suppressive function may counteract errant immune responses. The key to the stable epigenetic imprinting of Treg activity is a genetic element called Treg specific demethylated region (TSDR) in the *FOXP3* gene which is selectively demethylated in stable thymic-derived Tregs and hence, assures sustained expression of the Treg master transcription factor FOXP3. However, whether selective activation of the TSDR alone is sufficient to drive FOXP3 expression and possibly induce a Treg phenotype in primary conventional T cells, is so far unclear. We applied "epigenetic editing" to achieve a targeted demethylation of the TSDR using a fusion of the catalytically inactivated Cas9 (dCas) protein and the catalytic domain of the Ten-eleven translocation 1 (TET1) enzyme. Transient transfection of primary human naive and effector memory CD4+ T cells with a plasmid coding this construct and guide RNAs targeting the CpG motifs of the TSDR induced FOXP3 expression in a proportion of these cells. Resulting FOXP3+ cells were completely and selectively demethylated in all CpGs of the TSDR. Epigenetically edited T cell clones after extensive in vitro expansion maintained TSDR demethylation in the absence of the epigenetic editor, indicating that the induced TSDR demethylation is stable. Furthermore edited T cell clones displayed suppressive capacity in an in vitro suppression assay. Our results show that epigenetic editing is feasible in primary human T cells and that modification of only one element is sufficient for stable FOXP3 induction even in memory T helper cells.

WS.C5.01 Physiopathology of allergic disorders

WS.C5.01.01

FcγRIIA/CD32A-expressing platelets determine the severity of experimental anaphylaxis

F. Jönsson[1], *H. Beutier*[1], *B. Hechler*[2], *O. Godon*[1], *Y. Wang*[1], *C. M. Gillis*[1], *L. de Chaisemartin*[3], *A. Gouel-Chéron*[3], *S. Magnenat*[2], *L. Macdonald*[4], *A. Murphy*[4], *S. Chollet-Martin*[3], *D. Longrois*[3], *C. Gachet*[2], *P. Bruhns*[1], *NASA consortium;*
[1]*Institut Pasteur/Inserm, Paris, France,* [2]*EFS Grand Est/Inserm, Strasbourg, France,* [3]*Hôpital Bichat (AP-HP)/Inserm, Paris, France,* [4]*Regeneron Pharmaceuticals, New York, United States.*

Platelets are key regulators of vascular integrity; however, their role in anaphylaxis, a life-threatening systemic allergic reaction characterized by the loss of vascular integrity and vascular leakage, remains unknown. Anaphylaxis is a consequence of inappropriate cellular responses triggered by antibodies to generally inoffensive antigens, resulting in a massive mediator release and rapidly occurring organ dysfunction. Human platelets express receptors for IgG antibodies and can release potent mediators, yet their contribution to anaphylaxis has not been previously addressed in mouse models, probably because mice do not express IgG receptors on platelets.
We investigated whether platelets contribute to IgG anaphylaxis in human IgG receptor expressing mouse models and a cohort of patients suffering from drug-induced anaphylaxis. We found that platelet counts dropped immediately and dramatically at anaphylaxis induction, only when they expressed the human IgG receptor FcγRIIA/CD32A. Platelet depletion attenuated anaphylaxis whereas thrombocythemia drastically worsened its severity. FcγRIIA-expressing platelets were directly activated by IgG immune complexes *in vivo* and were sufficient to restore susceptibility to anaphylaxis in resistant mice. Serotonin released by activated platelets contributed to anaphylaxis severity. Data from a cohort of patients suffering from drug-induced anaphylaxis indicated that platelet activation was associated with anaphylaxis severity and that anaphylaxis occurrence was accompanied by a reduction in circulating platelet numbers.
Our findings identify platelets as critical novel players in IgG anaphylaxis and provide a rationale for the design of platelet-targeting strategies to attenuate the severity of anaphylactic reactions.
Funding: ERC, ANR-JCJC, Institut Pasteur, INSERM, Société Française d'Allergologie, Etablissement Français du Sang.

WS.C5.01.02

Novel role for the acute phase protein serum amyloid A in the initiation of type 2 immunity

U. Smole[1,2], *S. Lajoie*[2], *N. Gour*[2], *X. Xiao*[2], *J. Phelan*[2], *C. Köhler*[1], *N. Yao*[2], *L. Caraballo*[3], *E. Malle*[4], *S. Rosskopf*[1], *B. Kratzer*[1], *J. Chakir*[5], *A. Lane*[6], *W. Pickl*[1], *M. Wills-Karp*[2];
[1]*Institute of Immunology, Center for Pathophysiology, Infectiology, and Immunology, Medical University of Vienna, Vienna, Austria,* [2]*Department of Environmental Health and Engineering, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Institute of Immunological Research, Universidad de Cartagena, Cartagena, Colombia,* [4]*Gottfried Schatz Research Center, Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria,* [5]*Centre de Recherche, Institut Universitaire de Cardiologie et de Pneumologie de Québec, Université Laval, Québec, Canada,* [6]*Division of Rhinology and Sinus Surgery, Department of Otolaryngology – Head and Neck Surgery, Johns Hopkins School of Medicine,, Baltimore, MD, United States.*

In susceptible individuals otherwise innocuous environmental proteins such as allergens trigger the release of the type 2 promoting cytokine IL-33 that activates type 2 innate lymphoid cells (ILC2). Yet, the knowledge of how such allergens are sensed at mucosal surfaces is limited. We here identify the formyl-peptide receptor 2 (FPR2) and its endogenous ligand, the acute phase protein serum amyloid A1 (SAA1), as major drivers of house dust mite (HDM)-induced IL-33 release *in vitro* and *in vivo*. Mechanistically, this involved the dissociation of biologically inert SAA1 oligomers into monomers that induce epithelial IL-33 release. In mice, local inhibition of FPR2 in the lungs or *Saa1/2* deficiency abrogated HDM-induced airway hyperresponsiveness, IgE synthesis, bronchoalveolar lavage (BAL) eosinophilia, concomitant with reductions in Th2 cytokines, IL-13$^+$ ILC2s, and BAL IL-33 levels in allergen-exposed mice. This was dependent on SAA1 recognition of the cytosolic fatty acid binding protein (FABP) Der p 13 contained in HDM extract. Importantly our findings in mice translate to human allergic diseases. The FABP sensing pathway is upregulated in respiratory epithelial cells from chronic rhinosinusitis (CRS) patients resulting in increased IL-33 secretion associated with enhanced SAA1 monomer formation and FPR2 signaling. Taken together, we here report a novel mechanism of allergenicity which involves SAA1-facilitated allergen recognition via FPR2 leading to aberrant IL-33 release and type 2 responses. This novel paradigm allows for a new view on SAA1 as a potent driver of type 2 allergic immune responses at mucosal surfaces. Supported by the Austrian Science Fund FWF, NIH and ATS.

WS.C5.01.03

CD300c receptor co-stimulates IgE-mediated basophils activation and its expression is increased in cow´s milk allergic children

O. Zenarruzabeitia Belaustegi[1], *J. Vitallé*[1], *I. Terrén*[1], *A. Orrantia*[1], *I. Astigarraga*[1,2,3], *L. Dopazo*[1,2], *C. González*[4,3], *C. Tutau*[2], *L. Santos-Díez*[1,2], *P. Gamboa*[2], *A. Bilbao*[1,2], *F. Borrego*[1,5,6];
[1]*BioCruces Health Research Institute, Barakaldo, Spain,* [2]*Cruces University Hospital, Barakaldo, Spain,* [3]*University of the Basque Country, Leioa, Spain,* [4]*Basurto University Hospital, Bilbao, Spain,* [5]*Ikerbasque, Basque Foundation for Science, Bilbao, Spain,* [6]*Basque Center for Transfusion and Human Tissues, Galdakao, Spain.*

Basophils express high-affinity IgE receptors (FcεRI), which play an essential role in allergic diseases. To design new immunomodulatory therapies, it is important to characterize cell surface receptors that modulate IgE-mediated basophil activation threshold. We have analyzed the expression of CD300 receptors on human basophils and their implication in IgE-mediated basophil activation processes. Blood samples from healthy and allergic pediatric patients were collected. Peripheral blood mononuclear cells were obtained by density centrifugation, and phenotypical and functional studies were performed by flow cytometry. We demonstrate that basophils express the activating receptor CD300c, which is up-regulated in response to IL-3. CD300c works as a co-stimulatory molecule during IgE-mediated basophil activation, as shown by a significant increase in degranulation and cytokine production when basophils are activated through CD300c compared with the activation through the IgE/FcεRI axis alone. Co-ligation of FcεRI and CD300c increased intracellular calcium mobilization and phosphorylation of signalling intermediates evoked only by FcεRI ligation. Furthermore, we have observed that the expression of CD300c in allergic children is significantly increased compared to healthy controls, and that the intensity of expression correlates with the severity of the hypersensitivity symptoms. We conclude that CD300c could be considered a biomarker and therapeutic target in IgE-mediated allergic diseases as it seems to be involved in the modulation of IgE-mediated basophils activation.
Funding: ISCIII-Subdirección de Evaluación y Fomento de la Investigación-Fondo Europeo de Desarrollo Regional (FEDER) (Grant PI16/01223) and Sara Borrell postdoctoral contract (CD17/00128) to OZ. Fellowship FJGB17/003 to IT from Jesús de Gangoiti Barrera Foundation.

WS.C5.01.04

C5a receptor 1 activation on vacuolated eosinophils drives maladaptive immunity in experimental allergic asthma

A. V. Wiese[1], *K. M. Quell*[1], *F. Ender*[1], *T. Vollbrandt*[1], *P. König*[2], ***Y. Laumonnier***[1], *J. Köhl*[1];
[1]*ISEF, Lübeck, Germany,* [2]*Institute for Anatomy, Lübeck, Germany.*

The anaphylatoxin C5a plays an important role in the development of maladaptive Th2/Th17 immunity in experimental allergic asthma through the activation of C5a Receptor-1 (C5aR1). Here, we characterized a pulmonary highly vacuolated SiglecF$^+$CD11c$^+$ eosinophilic population (vEOS) that was localized in the lung, acted as an antigen-presenting cell that translocated to the mediastinal lymph nodes (mLN) and triggered T cell proliferation *in vitro*, as well as allergic asthma *in vivo*. EOS express high levels of C5aR1. Thus, we determined C5aR1 expression in vEOS, and its contribution to vEOS-driven allergic asthma using different adoptive transfer and allergen-induced allergic asthma models. We observed that C5aR1 is highly expressed in vEOS upon ovalbumin (OVA)-induced allergic asthma. In addition, vEOS expressed C5 and C5aR1 intracellularly. Interestingly, in *C5ar1*$^{-/-}$ mice, the recruitment of vEOS to mLN was significantly reduced and the upregulation of CD86 abrogated. Further, in co-cultures with OVA-TCR-transgenic T cells, the Th17 response induced by vEOS was increased in the absence of C5aR1.

Finally, transfer of house-dust-mite-pulsed *C5ar1*$^{-/-}$ vEOS into WT recipient mice resulted in low airway hyperresponsiveness (AHR) associated with a mixed Th1/Th2 response. Collectively, our data demonstrate that vEOS exert strong antigen-presenting properties, which are controlled by the C5a-C5aR1 axis. They activate T cells *in vitro* and *in vivo* to proliferate and differentiate into Th1 and Th2 effector cells. Such T cell activation is associated with the development of AHR, which is also controlled by activation of the C5a-C5aR1 axis. Thus, vEOS serve as additional antigen-presenting cells in experimental allergic asthma.

WS.C5.01.05
Single cell RNA-Seq identifies an exquisite pathogenic Th2 cell gene signature and pinpoints cellular metabolism as a major distinguishing factor

J. M. Coquet;
Karolinska Institutet, Solna, Sweden.

T helper cells are critical to the development of asthma. In particular, Th2 cells are the main protagonists in asthma, although other T helper cells subsets are known to participate and also regulate asthma severity. Although decades of research have elucidated a number of factors regulating T helper cell differentiation, gene expression profiles of T helper cells typically come from arrays of bulk cell populations from *in vitro*-differentiated cells. We performed single cell RNA-Sequencing on T helper cells from the airways of mice administered house dust mite antigens, in an attempt to obtain pure transcriptional profiles of T helper cell subsets, in particular the pathogenic Th2 cell population. Our results indeed clearly depict populations of Th2, Treg and Th1 cells. In addition, we characterize a population of type I IFN-responding cells and a population of activated T helper cells, expressing mRNA for ribosomal proteins. Th2 cells indeed expressed *Gata3, Il4, Il5 and Il13* as expected, but were also highly enriched for the expression of approximately 100 other genes, many of whose functions are not typically associated with Th2 cells. Pathway analysis identified metabolic processes as the major point of difference between Th2 cells and other T helper cells in the airways, in particular, those relating to fatty acid oxidation and synthesis. Taken together, our data shows that Th2 cells differ substantially from other T helper cells in a mouse model of asthma, with cellular metabolism being a major point of difference. Work characterizing human Th2 cells is ongoing.

WS.C5.01.06
Notch signaling in CD4^{+} T cells supports lymph node egress of Th2 cells in allergic airway inflammation

I. Tindemans[1], *A. KleinJan*[1], *M. J. de Bruijn*[1], *M. Lukkes*[1], *A. van den Branden*[1], *W. F. van Ijcken*[1], *D. Amsen*[2], *R. Stadhouders*[1], *R. W. Hendriks*[1];
[1]*Erasmus MC, Rotterdam, Netherlands,* [2]*Sanquin, Amsterdam, Netherlands.*

Background: Allergic asthma is characterized by a T helper-2 (Th2) response to inhaled allergens. Previous experiments showed that Notch signaling controls Th2 differentiation through direct induction of Gata3. However, the role of Notch signaling during Th2-mediated allergic airway inflammation (AAI) *in vivo* is currently unclear.
Methods: We used a house-dust mite (HDM)-driven model to investigate the capacity of Notch1 and Notch2-deficient T cells to induce AAI. The role of Notch signaling *in vitro* and *in vivo* was studied using Notch-deficient ovalbumin-specific T cells. We performed transcriptome analysis of T cells to identify genes that are controlled by Notch signaling in the context of AAI.
Results: In the HDM-treated groups, WT animals developed AAI, but conditional Notch1/Notch2 double KO animals failed to develop eosinophilic inflammation, and Th2 cytokines or serum IgE were not induced. When conditional Notch KO mice which concomitantly expressed a CD2-Gata3 transgene were treated with HDM, only a partial enhancement of Th2 inflammation was observed. Surprisingly, we found that Notch signaling is not required for the induction of Th2 cell differentiation. However, during the allergen challenge phase Notch-deficient T cells did not cause AAI, as they accumulated in draining lymph nodes and did not efficiently migrate to the lung. Transcriptome comparisons of Notch-deficient and WT Th2 cells from lymph nodes revealed 692 differentially expressed genes, including genes involved in cell migration and adhesion.
Conclusion: Notch signaling in T cells is crucial during Th2-mediated AAI in a HDM-driven asthma model, likely by mediating lymph node egress of Th2 cells.

WS.C5.02 Immunotherapy of allergic disorders

WS.C5.02.01
Selective elimination of allergen-specific B lymphocytes with chimeric protein-engineered molecules

N. R. Ralchev[1], *N. Mihaylova*[1], *N. Kerekov*[1], *A. Tchorbanov*[1,2];
[1]*Laboratory of Experimental Immunology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria,* [2]*National Institute of Immunology, Sofia, Bulgaria.*

Introduction: Der p1 is allergenic molecule of Dermatophagoides pteronyssinus (Dpt) which causes house dust mite (HDM) allergy. The pathological Der p1-specific B cells produce allergen-specific IgE antibodies that mediate most of the hypersensitivity allergic reactions. It may be possible to influence Der p1- specific B cells in mouse model of HDM allergy by administration of chimeric molecule, containing a monoclonal antibody against the inhibitory B-cell receptor FcγRIIb coupled to a B and a T cell epitope-carrying peptides from the Der p1 allergen. Co-crosslinking of the immunoglobulin receptors and FcγRIIb by this molecule is expected to deliver strong suppressive signal selectively silencing these B cells and the subsequent allergic response. Materials and Methods: protein engineering, FACS, animal model, immunohistochemistry, histology. Results: A protein engineered chimeric molecule has been constructed, which binds Der p 1 specific B-cells via their BCR and suppresses selectively the production of anti - Der p 1 antibodies via FcγRIIb. The synthetic peptide Der p1 p52-71 and an anti-FcγRIIb monoclonal antibody were used for the construction of Der p1 chimera. The functional effects of engineered antibodies were analyzed *in vitro*. An animal model of HDM allergy has been developed. Significant inhibition of allergen-specific proliferation and reduction of DerP1 - IgE antibody production were observed after treatment of splenocytes from sick mice with DerP1-peptide chimera. Conclusions: The present study explores a different approach for preventing pathological allergen-specific IgE antibody production. Our data show that the allergic immune response can be silenced by specific targeting the pathological B cells.

WS.C5.02.02
Sulfated Non-Anticoagulant Heparin (S-NACH) blocks allergen induced asthma manifestations in mice by modulating the IL-4/STAT6/GATA-3 pathway harboring a therapeutic potential for allergic asthma

M. A. Ghonim[1], *J. Wang*[1], *S. Ibba*[1], *K. Pyakurel*[1], *H. Luu*[1], *I. Benslimane*[1], *S. Mousa*[2], *H. Boulares*[1];
[1]*Louisiana State University Health Sciences Center, New Orleans, LA, United States,* [2]*Albany College of Pharmacy and Health Sciences, the Pharmaceutical Research Institute, Albany, NY, United States.*

Background: The efficacy of heparins and Low-MW-heparins (LMWH) against human asthma has been known for decades. However, the clinical utility of these compounds has been hampered by their anti-coagulant properties. Much effort has been made to harness the anti-inflammatory of LMWH but so far none have gone to be used as therapy for asthma. Sulfated-Non-Anticoagulant Heparin (S-NACH) is an ultra-LMWH with no systemic anticoagulant effects. The present study explored the potential of S-NACH in blocking allergic asthma. ***Methods***: Acute and chronic ovalbumin-based mouse models of asthma, splenocytes, and a lung epithelial cell line were used. Mice were challenged to aerosolized ovalbumin. Mice were administered S-NACH or saline 30 min after each ovalbumin challenge. ***Results***: S-NACH administration in mice promoted a robust reduction in airway eosinophilia, mucus production and airway hyperresponsiveness even after chronic repeated challenges with ovalbumin. Such effects were linked to a suppression of the Th2 cytokines IL-4/IL-5/IL-13/GM-CSF and ovalbumin-specific IgE without any effect on IFN-γ. S-NACH also reduced lung fibrosis in chronically ovalbumin-exposed mice. These protective effects of S-NACH may be attributed to modulation of the IL-4/JAK signal transduction pathway through an inhibition of STAT6 and a subsequent inhibition of GATA-3 and inducible NO synthase expression. S-NACH treatment also reduced the basal expression of the two isoforms of arginase Arg1 and Arg2 in lung epithelial cells. ***Conclusions***: our results demonstrate that S-NACH may represent an opportunity to take advantage of the well-known anti-asthma properties of heparins and LMWH while bypassing the risk of bleeding complications.

WS.C5.02.03
Targeting PDE3 Abrogates the Hallmarks of Asthma in Asthma Models

A. KleinJan[1], *J. Beute*[1], *K. Ganesh*[1], *V. Bos*[1], *M. Lukkes*[1], *M. v. Nimwegen*[1], *S. Hockman*[2], *V. Manganiello*[2], *R. Hendriks*[1];
[1]*Erasmus MC, Rotterdam, Netherlands,* [2]*NIH, Bethesda, United States.*

Objective: Case reports related to status asthmaticus showed that enoximone, which is a smooth muscle relaxant that inhibits PDE3, is beneficial and lifesaving. Off label use of enoximone has steroid sparing properties. From PDE3 inhibition it is not known that it has anti-inflammatory effects. Here, we investigated the pathophysiological role and disease modifying effects of PDE3 in acute and chronic house dust mite (HDM)-driven asthma mouse models. Methods: Bronchial hyperreactivity (BHR) and allergic airway inflammation (AAI) and airway remodeling was investigated by Buxco, histology and flowcytometry in chronic HDM-driven asthma models. The pathophysiological role was studied by intervention of PDE3 by transgenic mice lacking PDE3 or wild type mice treated with the PDE3 inhibitor enoximone when AAI was already established. Mucosal barrier function was investigated by measuring albumin leakage and the presence of albumin in lung tissue. Results: Mice lacking PDE3 or treated with the PDE3 inhibitor enoximone showed reduced BHR; reduced AAI as measured by BAL eosinophilia and Th2-cell cytokine profile and showed improved mucosal barrier function when compared to wild type control mice in the HDM-driven asthma models. Having established HDM driven AAI, reduced BHR, reduced smooth muscle mass and reduced mucus hypersecretion depict the role of enoximone in abrogating airway remodelling. In addition, diminished inflammatory cells and epithelial mast cells depicted its broader anti-inflammatory effects of PDE3 inhibition when intervention with enoximone or placebo starts after already established HDM driven AAI. Conclusion: Taken together this study prove the pathophysiological role of PDE3 in asthma models

WS.C5.02.04

Neutrophils negatively regulate T_H2 inflammation in allergic airways disease by limiting G-CSF driven monocytosis and ILC2 cytokine production

***D. F. Patel**, T. Peiro, J. Vuononvirta, S. Akthar, S. A. Walker, L. G. Gregory, C. M. Lloyd, R. J. Snelgrove;*
Imperial College London, London, United Kingdom.

Neutrophils are critical components of the body's anti-microbial response, yet an over-exuberant neutrophilic inflammation has traditionally been implicated in the pathology of chronic diseases. Increasingly, however, it is recognized that neutrophils are not just indiscriminate killers but are able to elegantly and specifically shape many facets of the elicited immune response. Largely circumstantial evidence has suggested a prominent role for neutrophils in the pathophysiology of severe asthma, but their precise contribution to disease progression is poorly understood. In a house dust mite model (HDM) of allergic airways disease (AAD), we demonstrate that neutrophil depletion unexpectedly resulted in exacerbated T_H2 inflammation, with increased numbers of T_H2 $CD4^+$ T-cells and type 2 innate lymphoid cells (ILC2s) and elevated levels of allergen-specific IgE and IgG1 antibodies. This augmented T_H2 inflammation in neutrophil depleted mice was preceded by an early increase in levels of ILC2-derived IL-5 and IL-13 and numbers of monocyte-derived dendritic cells (MoDCs) and ensuing antigen presentation. Central to the exacerbated T_H2 inflammation in neutrophil-depleted mice was a striking increase in G-CSF levels, which drove an expansion on monocyte progenitors in the bone marrow and T_H2 cytokine production by ILC2s in the airways. In conclusion, we demonstrate that neutrophil depletion during AAD results in profound perturbations in the G-CSF axis and an ensuing monocyte and ILC2 driven exacerbation in T_H2 inflammation. These studies reveal a novel role for neutrophils in negatively regulating monocyte and ILC2 function and highlight potential complexities of targeting neutrophils in animal models and within the clinic.

WS.C5.02.05

NOD/SCID-mice engrafted with human allergen-specific $CD4^+$ T cells develop allergic airway responses without signs of GvHD

***C. Vizzardelli**[1], F. Zimmann[1], M. Gindl[1], B. Nagl[1], C. Kitzmüller[1], U. Vollmann[1], S. Tangermann[2], L. Kenner[2,3,4], B. Bohle[1];*
[1]Center of Pathophysiology, Infectiology and Immunology, Medical University Vienna, Vienna, Austria, [2]Unit of Laboratory Animal Pathology, University of Veterinary Medicine Vienna, Vienna, Austria, [3]Department of Experimental and Laboratory Animal Pathology, Medical University of Vienna, Vienna, Austria, [4]Ludwig Boltzmann Institute for Cancer Research, Vienna, Austria.

Introduction: We have successfully employed non-obese diabetic severe-combined-immunodeficient $\gamma c^{-/-}$ (NSG) mice engrafted with PBMC from allergic patients as *in vivo* model of respiratory allergy. Allergic airway inflammation in this model is mainly mediated by allergen-specific $CD4^+$ T-cells. As their frequency in PBMC of allergic individuals is very low and $CD8^+$ T-cells may trigger graft-versus-host-disease (GvHD) we sought to improve this model by employing T-cell lines (TCL) enriched for allergen-specific $CD4^+$ T-cells.
Methods: Betv1-specific TCL were established from birch pollen-allergic patients. The ratio of CD4/CD8 T-cells was analysed by flow cytometry. Allergen-reactivity was confirmed in proliferation assays. T-cell-specificities were identified with overlapping synthetic 12-mer peptides. Allergen-induced cytokine responses were assessed with qPCR. Betv1-specific TCL plus autologous antigen-presenting cells were injected intraperitoneally. After 17 days engraftment was assessed by flow cytometry. Airway hyperresponsiveness (AHR) and bronchial inflammation were analyzed after intranasal challenges with allergen or PBS.
Results: After injection of TCL harbouring $CD4^+$ T-cells reactive with various epitopes of Betv1, $CD4^+$ T-cells were detected in cell suspensions of lungs and spleens. $CD8^+$ T-cells did not expand *in vivo*. Mice challenged with allergen showed significantly higher AHR and larger numbers of eosinophils, neutrophils and basophils in bronchoalveolar fluids than those challenged with PBS. Lung histology revealed peribronchial inflammation in mice challenged with allergen. No perivascular inflammation indicative of GvHD was observed.
Conclusion: NSG-mice engrafted with allergen-specific $CD4^+$ TCL develop allergic airway responses without GvHD. This improved *in vivo* model may be useful in the assessment of novel therapeutic allergy vaccines targeting allergen-specific T-lymphocytes.

WS.C5.02.06

Induction of *in vitro* cross-tolerance in birch pollen-allergic patients with associated food allergies by use of tolerogenic dendritic cells

***P. V. Rostan**[1], E. Graulich[1], V. K. Raker[1], A. Wangorsch[2], S. Scheurer[2], K. Steinbrink[1];*
[1]Department of Dermatology, Division for Experimental and Translational Research, University Medical Center, Mainz, Germany, [2]Paul-Ehrlich-Institut, Molecular Allergology, Langen, Germany.

Birch-pollen allergy is one of the most prevalent allergic diseases in Northern Europe. Among birch-pollen allergic patients approximately 70% experience secondary food allergy e.g. to hazelnuts, carrots, apples or others. Increasing evidence has shown that this oral allergy syndrome (OAS) is mediated by both, IgE and T cell cross-reactivity to proteins of the pathogen related protein family 10 (PR-10) which are homologous to the major birch pollen allergen Bet v 1.0101 (e.g. Cor a 1.0401 (hazelnut)). In previous studies, we found that human tolerogenic interleukin-10-modulated dendritic cells (IL10-DC) induce anergic regulatory T cells (iTregs) exhibiting a high suppressive capacity. Therefore, we investigate the potential of IL10-DC to induce allergen-specific and cross-reactive iTregs *in vitro* in allergic patients with proven birch pollen and cross-reactive hazelnut allergies. Stimulation with Bet-v-1-loaded mature DC led to pronounced Bet-v-1-specific effector T cell proliferation whereas Bet-v-1-pulsed IL10-DC induced hypoproliferative T cells, indicating the generation of Bet-v-1-specific iTregs. However, restimulation of Bet-v-1-specific iTregs with syngeneic Bet-v-1- or Cor-a-1-loaded mDC resulted in a significantly increased or similar T cell proliferation, respectively, compared to Bet-v-1-specific effector T cells. In further experiments, we want to characterize the induced iTreg populations and explore their suppressive capacity by use of *in vitro* and *in vivo* suppressor assays. The goal of our study is the development of novel therapeutic approaches for type I allergies, including (food) cross-allergies, using tolerogenic IL-10-modulated DC for tolerance induction.
This study is financially supported by the German Research Foundation (DFG).

WS.C6.01 Acquired immunity crosstalk with inflammation

WS.C6.01.01

hIPSC-derived dendritic cells identify IRF5 as a crucial regulator that exacerbates inflammatory cytokine production during Influenza A virus infection

***J. L. Forbester**[1,2], M. Clement[1], A. Yeung[2], P. Sabberwal[1], M. Marsden[1], G. Dougan[3,2], I. Humphreys[1,2];*
[1]School of Medicine, Cardiff, United Kingdom, [2]Wellcome Trust Sanger Institute, Cambridge, United Kingdom, [3]University of Cambridge, Cambridge, United Kingdom.

IRF5 is a transcription factor expressed by myeloid cells, which plays important roles in Toll-like receptor (TLR), IFN and virus-induced signalling pathways. Human induced pluripotent stem cell (hIPSC)-derived cellular systems provide an exciting potential route towards the analysis of human cell function in a genetically defined environment, and may help elucidate complex mechanisms underlying host-pathogen interactions.
Here, we use iPSC technology to define the role of IRF5 in human myeloid cells in response to Influenza A virus (IAV). We differentiate healthy control hIPSCs, and hIPSCs with biallelic mutations in *IRF5*, generated using CRISPR/Cas9 technology, into iPS-Dendritic Cells (iPS-DCs). iPS-DCs expressed markers of $CD141^+$ myeloid DCs, and iPS-DCs generated from *IRF5*-/- iPSCs produce significantly less IL-6 in response to IAV infection, and also after stimulation with various TLR ligands. To confirm this phenotype was not due to off-target effects, we used TALEN engineering to integrate a functional copy of IRF5 back into the *IRF5*-/- iPSC line. The IL-6 response to IAV in iPS-DCs derived from $IRF5^{Comp}$ iPSCs was restored. To examine the role of IRF5 during *in vivo* infection, we challenged *Irf5*-/- mice with IAV. *Irf5*-/- mice experienced reduced pathology after IAV challenge. Despite having no impact on virus replication, *Irf5*-/- mice exhibited ameliorated pathology which was associated with reduced inflammatory cytokine production in the lung.
Therefore, we demonstrate in both human and murine model systems that IRF5 acts as a regulator for myeloid cell inflammatory cytokine production during IAV infection, and drives immune-mediated viral pathogenesis.

WS.C6.01.02

SOCS1 and SOCS3 target IRF7 degradation to suppress TLR7- mediated type I interferon production of human plasmacytoid dendritic cells

***C. Yu**[1], W. Peng[1], M. Schlee[2], W. Barchet[2], A. M. Eis-Hübinger[3], W. Kolanus[4], M. Geyer[5], S. Schmitt[5], F. Steinhagen[6], J. Oldenburg[7], N. Novak[1];*
[1]Department of Dermatology and Allergy, Bonn, Germany, [2]Institute of Clinical Chemistry and Pharmacology, Bonn, Germany, [3]Institute of Virology, Bonn, Germany, [4]Department of Molecular Immune and Cell Biology, Life and Medical Sciences (LIMES) Institute, Bonn, Germany, [5]Institute of Structural Biology, Bonn, Germany, [6]6Department of Anesthesiology and Intensive Care Medicine, Bonn, Germany, [7]Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany.

Introduction: Type I interferon (IFN) production of plasmacytoid dendritic cells (pDCs) triggered by toll-like receptor (TLR) activation is essential for antiviral responses and autoimmune reactions. The mechanism how SOCS proteins regulate TLR7-mediated type I IFN production in pDCs was largely unknown. Therefore, we aimed to investigate the role of SOCSs in TLR7- type I IFN pathway in pDCs.
Materials and Methods: The regulation of TLR7-signaling and type I IFN production by SOCS1/3 in fresh human pDCs and pDC cell line were evaluated. HEK 293 cells overexpressing SOCS1/3 as well as their mutants were conducted for signaling investigation.
Results: TLR7 activation induced the expression of SOCS1 and SOCS3 in human pDCs, SOCS1 and SOCS3 intensely dampened TLR7-mediated type I IFN production through targeting interferon regulatory factor (IRF) 7, a pivotal transcription factor of the TLR7 pathway. Further study revealed SOCS1 and SOCS3 bound IRF7 through the SH2 domain to promote its proteasomal degradation by lysine 48-linked polyubiquitination.
Conclusion: Our results demonstrate that SOCS1/3-mediated ubiquitination and degradation of IRF7 directly regulates TLR7 signaling and type I IFN production in pDCs. This mechanism might be targeted by therapeutic approaches to either enhance type I IFN production in antiviral treatment or decrease type I IFN production in the treatment of autoimmune diseases (The paper based on the study was recently accepted by the Journal of Immunology).

WS.C6.01.03

The dialogue between mast cells and B lymphocytes may precipitate atherosclerosis complications

A. Loste[1,2], *K. Guedj*[3], *M. Clement*[3], *S. Delbosc*[3], *M. Morvan*[3], *G. Even*[3], *A. Gaston*[3], *M. Arock*[4], *A. Eggel*[5], *J. B. Michel*[3], *G. Caligiuri*[3,6], *A. Nicoletti*[3,2], *M. Le Borgne*[3,2];
[1]*INSERM U1148, Paris, France,* [2]*Université Paris Diderot, Université Sorbonne Paris Cité, Paris, France,* [3]*INSERM U1148 "Laboratory for Vascular Translational Science", Paris, France,* [4]*CNRS U8147 "Laboratoire de biotechnologies et pharmacologie génétique appliquée", Cachan, France,* [5]*Institute of Immunology, University of Bern, Bern, Switzerland,* [6]*Assistance Publique Hôpitaux de Paris, Hôpital Bichat, Paris, France.*

Atherosclerosis leads to cardiovascular events such as myocardial infarction or stroke. The immune effectors that are recruited to the atheromatous arteries are suspected to accelerate atherosclerosis complications. Notably, mast cells (MCs) and germinal center (GC) B cells accumulate in atheromatous arteries. Also, serum IgE levels are associated with the severity of arterial diseases. We hypothesize that the MC-B cell-IgE axis could form a local amplification loop that could precipitate the evolution of atherosclerotic lesions.
To test this hypothesis, we compared paired blood and arterial samples from patients with arteries displaying complicated atheroma (ACA) or having healthy arteries (HA).
We observed that (1) the IgE levels were higher in arteries from ACA than from HA, (2) the GC B cells and plasma cells of ACA were absent from the paired patient blood, and (3) the ACA GC stained positive for IgE. These results suggest that B cells differentiate into IgE-producing cells, locally, in diseased arteries. In addition, ACA MCs are activated, covered by IgE, and located in the vicinity of GC. MCs could therefore be activated by locally produced IgE and could also interact with B cells. Importantly, IgE-antigen complexes present only in conditioned medium from ACA induced the release of IL-4 by human MCs. IL-4 is a cytokine required for B cell class switching toward IgE.
Altogether, our results identify a local loop wherein IgE would trigger the production of IL-4 by MCs, which in turn would promote the IgE-class switching of maturating GC B cells.
Support: DHU FIRE, CORDDIM

WS.C6.01.04

A role for DPP4 in T cell-mediated vascular inflammation and Atherosclerosis

J. Zhong[1], *L. Duan*[1], *C. Xia*[1], *J. Varghese*[2], *G. Mihai*[3], *X. Rao*[1], *S. Rajagopalan*[1];
[1]*Case Western Reserve University, Cleveland, United States,* [2]*Ohio State University, Columbus, United States,* [3]*Harvard Medical School, Boston, United States.*

Dipeptidyl peptidase IV (DPP4), an ubiquitously expressed peptidase, plays an important role in regulating postprandial blood glucose by degrading incretin hormones such as GLP-1 and GIP. In this study, we provided evidence showing that hematopoietic cell derived DPP4 regulates vascular inflammation and atherosclerosis development by controlling T cell migration. T cells isolated from human peripheral blood, especially CD4+ T cells express high levels of DPP4. The expression of DPP4 on circulating CD4+ T cells was significantly increased in patients with atherosclerosis and positively correlated with plasma levels of non-HDL cholesterol and triglyceride. Hematopoietic deficiency of DPP4 protected *Ldlr*$^{-/-}$ mice from high fat diet-induced atherosclerosis progression, accompanied by a reduced T cell infiltration in aortic plaque. Chimeric mice with *Dpp4*$^{-/-}$ bone marrows showed an improved response to oral glucose challenge and a much less expression of DPP4 in aorta, suggesting bone marrow derived cells are an important source for DPP4 within the circulation and the aorta. Transwell assay showed migrated T cells had higher expression of DPP4 compared to unmigrated T cells. Both *in-vitro* and *in-vivo* migration assays suggest *Dpp4*$^{-/-}$ T cells had a reduced migratory ability. By using enzymatic DPP4 inhibitor, linagliptin, we found both enzymatic and non-enzymatic actions of DPP4 are involved in the regulation of T cell migration. Taken together, our results suggest that the expression of DPP4 on hematopoietic cells promotes vascular inflammation and atherosclerotic progression by regulating T cell migration. **Acknowledgement** This work was supported by grants from AAI (CIIF-8745), AHA (17GRNT33670485), and Boehringer Ingelheim (IIS2015-10485).

WS.C6.01.05

VISTA expression by microglia decreases during inflammation and is differentially regulated in CNS diseases

M. Borggrewe[1], *C. Grit*[1], *W. F. den Dunnen*[1], *S. M. Burm*[2,3], *J. J. Bajramovic*[2], *R. J. Noelle*[4], *B. J. Eggen*[1], *J. D. Laman*[1];
[1]*University Medical Center Groningen, Groningen, Netherlands,* [2]*Biomedical Primate Research Centre, Rijswijk, Netherlands,* [3]*Genmab B.V., Utrecht, Netherlands,* [4]*Geisel School of Medicine at Dartmouth, Lebanon, NH, United States.*

V-type immunoglobulin domain-containing suppressor of T-cell activation (VISTA) is a negative checkpoint regulator (NCR) involved in inhibition of T cell-mediated immunity. Expression changes of other NCRs (PD-1, PD-L1/L2, CTLA-4) during inflammation of the central nervous system (CNS) were previously demonstrated, but VISTA expression in the CNS has not yet been explored. Here, we report that in the human and mouse CNS, VISTA is most abundantly expressed by microglia, and to lower levels by endothelial cells. Upon TLR stimulation, VISTA expression was reduced in primary neonatal mouse and adult rhesus macaque microglia *in vitro*. In mice, microglial *VISTA* expression was reduced after lipopolysaccharide (LPS) injection, during experimental autoimmune encephalomyelitis (EAE), and in the accelerated aging *Ercc1*$^{\Delta/-}$ mouse model. After LPS injection, decreased *VISTA* expression in mouse microglia was accompanied by decreased acetylation of lysine residue 27 in histone 3 in both its promoter and enhancer region. ATAC-sequencing indicated a potential regulation of *VISTA* expression by Pu.1 and Mafb, two transcription factors crucial for microglia function. Finally, our data suggested that VISTA expression was decreased in microglia in multiple sclerosis lesion tissue, whereas it was increased in Alzheimer's disease patients. We are currently analysing VISTA expression in different types of MS lesions in detail. This study is the first to demonstrate that in the CNS, VISTA is expressed by microglia, and that VISTA is differentially expressed in CNS pathologies.

WS.C6.01.06

CLEC12A regulates neutrophil functioning in Rheumatoid arthritis

L. Whitehead[1], *P. Morvay*[1], *P. Redelinghuys*[1], *I. Dambuza*[1], *S. Ansboro*[1], *A. J. Roelofs*[1], *D. M. Reid*[1], *J. A. Willment*[1], *C. De Bari*[1], *I. B. McInnes*[2], *G. D. Brown*[1];
[1]*Institute of Medical Sciences, Aberdeen, United Kingdom,* [2]*Institute of Infection, Immunity and Inflammation, Glasgow, United Kingdom.*

Rheumatoid arthritis (RA) is a complex autoimmune disease characterised by progressive joint deformity and systemic co-morbidities. Myeloid cells such as neutrophils can be found in abundance in the synovial fluid of patients and RA animal models where they release neutrophil extracellular traps (NETs), reactive-oxygen species (ROS) and other inflammatory mediators aiding the progression of joint destruction. CLEC12A (or MICL, Myeloid inhibitory C-type lectin), an inhibitory receptor expressed on myeloid cells, has previously been linked to the control of joint sterile inflammation through recognition of monosodium urate microcrystals (MSU). In a murine model of RA, *Clec12a* knock-out (KO) mice exhibit increased joint inflammation, bone erosion and an amplified influx of neutrophils into the synovium. Adoptive transfer of *Clec12a* KO neutrophils into wild-type (WT) mice can transiently recapitulate the KO mice inflammatory defect. Functionally, *Clec12a* KO neutrophils release increased ROS and NETs in response to stimulation *in vitro*. Inhibition of NETosis *in vivo,* via treatment with BB-CL-Amidine, reduced joint inflammation in *Clec12a* KO mice. While we did not find any association of polymorphisms in *CLEC12A* with disease, a subset of RA patients presented with autoantibodies against CLEC12A in their serum. Importantly, administration of anti-CLEC12A antibodies to WT mice induces the same exacerbation of inflammation seen in the KO mice. Together these data suggest a novel mechanism whereby autoantibodies may interfere with an inhibitory pathway regulating neutrophil responses in the initiation of RA.

WS.C6.02 New mediators in inflammation and its resolution

WS.C6.02.01

PPAR-γ modulates macrophage response to lipopolysaccharide and glucocorticoids

M. Heming[1], *S. Gran*[1], *S. Jauch*[1], *L. Fischer-Riepe*[1], *A. Russo*[1], *L. Klotz*[2], *S. Hermann*[3], *M. Schaefers*[3], *J. Roth*[1], ***K. Barczyk-Kahlert***[1];
[1]*Institute of immunology, University of Muenster, Muenster, Germany,* [2]*Department of Neurology, University Hospital Muenster, Muenster, Germany,* [3]*European Institute for Molecular Imaging, University of Muenster, Muenster, Germany.*

We could previously show that treatment of monocytes with glucocorticoids (GC) does not cause a global suppression of monocytic effector functions, but rather induces differentiation of a specific anti-inflammatory phenotype. However, a relationship between GC treatment and PPAR-γ expression in macrophages has not been investigated so far. We established ER-Hoxb8-immortalized bone marrow-derived macrophages from *Pparg*$^{fl/fl}$ (WT) and *LysM-Cre Pparg*$^{fl/fl}$ (PPAR-γ KO) mice in this study. The ER-Hoxb8 system allows the generation of a homogeneous and well-defined population of resting macrophages. We could show that the loss of PPAR-γ resulted in delayed kinetic of differentiation of monocytes into macrophages and could also demonstrate that CD38 expression in macrophages depends on PPAR-γ. As expected, PPAR-γ KO macrophages displayed an increased pro-inflammatory phenotype upon long-term LPS stimulation and showed impaired phagocytosis compared to WT cells. GC treatment of macrophages led to the upregulation of PPAR-γ expression. However, there were no differences in GC-induced suppression of cytokines between both cell types. Intriguingly, GC treatment resulted in an increased *in vitro* migration only in PPAR-γ KO macrophages. Performing *in vivo* cell tracking in a cutaneous granuloma mouse model, we could confirm that PPAR-γ KO, but not WT, macrophages treated with GC showed increased recruitment to the site of inflammation. In conclusion, we could demonstrate that PPAR-γ exerts anti-inflammatory activities and shapes macrophage functions. Moreover, we identified a molecular link between GC and PPAR-γ and could show for the first time that PPAR-γ modulates GC-induced migration in macrophages.

WS.C6.02.02
Anti-inflammatory microRNA-146a protects mice from diet-induced metabolic disease

M. C. Runtsch[1,2], *M. Nelson*[2], *R. M. O'Connell*[2];
[1]*Trinity College, Dublin, Ireland,* [2]*University of Utah, Salt Lake City, Utah, United States.*

Identifying regulatory mechanisms that influence inflammation in metabolic tissues, including adipose and liver, are critical to developing treatments for metabolic diseases such as obesity and diabetes. Here, we investigated the protective role of microRNA-146a (miR-146a) during diet-induced obesity (DIO). This microRNA has been shown to be reduced in obese and type 2 diabetic patients and to regulate inflammation in other contexts. Results revealed that miR-146a-/- mice fed a high-fat diet (HFD) have increased weight gain and adiposity, exaggerated adipocyte hypertrophy, liver steatosis, and diabetes compared to WT controls. Pro-inflammatory gene expression, NFκB activation, and crown-like structure accumulation increased in adipose tissue in the absence of miR-146a, indicating that this microRNA regulates inflammation in metabolic tissues. miR-146a was highly expressed in the stromal vascular fraction (SVF) of adipose tissue, suggesting a role for this microRNA in adipose immune cells. *Ex vivo* RNA-sequencing of adipose tissue macrophages (ATMs) further demonstrated a requirement for miR-146a in regulating not only inflammatory pathways, but also cellular metabolism during obesity, a role which has not been previously identified. We determined that miR-146a regulates inflammation and cellular respiration in macrophages through its mRNA target *Traf6*. Altogether, these data demonstrate important functions for miR-146a in preventing obesity and metabolic disease by regulating immunometabolic pathways within macrophages.
Training for this project was funded by an NIH T32 in Metabolism and Diabetes, awarded to M.R.

WS.C6.02.03
IFNb is a novel effector cytokine in resolving inflammation

A. Ariel[1], *S. K. Satyanarayanan*[1], *S. Soboh*[1], *S. Butenko*[1], *J. Saadi*[1], *A. Kassis*[1], *N. Peled*[1], *N. Sher*[2], *S. Schif-Zuck*[1];
[1]*University of Haifa, Haifa, Israel,* [2]*The Tauber Bioinformatics Research Center, University of Haifa, Haifa, Israel.*

The engulfment of apoptotic leukocytes (efferocytosis) by macrophages during the resolution of inflammation is essential for tissue homeostasis and results in macrophage reprogramming/immune-silencing. However, a distinct subset of resolution phase macrophages loss their phagocytic potential, and hence were termed satiated macrophages. Here, we show using an unbiased RNA-Seq analysis that satiated macrophages express a distinct IFNβ-related signature. Unexpectedly, we found peritoneal IFNβ levels peaked during the onset as well as the resolution phase of peritonitis. Consequently, we determined IFNβ limited the onset of neutrophilic inflammation by enhancing PMN apoptosis. Moreover, IFNβ enhanced macrophage efferocytosis and reprogramming to a pro-resolving phenotype. These findings indicate for the first time that IFNβ is a key effector cytokine in resolving inflammation.

WS.C6.02.04
CXCL9(74-103) reduces neutrophil recruitment and joint inflammation in experimental arthritis

D. Boff[3], ***H. Crijns***[2], *R. Janssens*[2], *V. Vanheule*[2], *G. B. Menezes*[3], *S. Macari*[4], *T. A. Silva*[4], *F. A. Amaral*[1], *P. Proost*[2];
[1]*Instituto de Ciencias Biologicas, UFMG, Belo Horizonte, Brazil,* [2]*Rega Institute, KU Leuven, Leuven, Belgium,* [3]*Centro de Biologia Gastrointestinal, UFMG, Belo Horizonte, Brazil,* [4]*Faculdade de Odontologia, UFMG, Belo Horizonte, Brazil.*

The effect of the C-terminal CXCL9-derived peptide, CXCL9(74-103), on joint inflammation was investigated in a murine model of antigen-induced arthritis (AIA) in male C57BL/6J mice. Simultaneous intravenous injection of CXCL9(74-103) with a tibiofemoral challenge with methylated bovine serum albumin (mBSA) as antigen in mice immunized with mBSA, resulted in a reduced accumulation of leukocytes, mainly neutrophils, in the synovial cavity. Periarticular tissue levels of the neutrophil-attractant chemokines (CXCL1, CXCL2 and CXCL6) and interleukin (IL)-6 were decreased in mice treated with CXCL9(74-103) compared to non-treated AIA mice. In addition, joint and cartilage damage were substantially reduced upon CXCL9(74-103) treatment. CXCL9(74-103) competes with CXCL6 and CCL3 for binding to glycosaminoglycans *in vitro*. Confocal microscopy allowed to visualize rapid binding of CXCL9(74-103) to blood vessels in joints. Delayed (up to 6 hours) treatment with CXCL9(74-103) still reduced neutrophil accumulation in the joint, but did not affect chemokine and IL-6 concentrations. Further delay of treatment failed to affect cell recruitment and chemokine and cytokine levels. Taken together, we show that a chemokine-derived peptide controlled the massive accumulation of neutrophils in the joint of AIA mice through inhibition of the interaction between chemokines and glycosaminoglycans and diminished tissue damage. This research was supported by KU Leuven, the Hercules Foundation, CNPq Brazil and FWO Vlaanderen. H.C. holds a SB PhD fellowship of the FWO Vlaanderen, Belgium.

WS.C6.02.05
Inhibition of neutrophil extravasation with a CXCL9-derived glycosaminoglycan-binding peptide

P. Proost[1], *V. Vanheule*[1], *A. Mortier*[1], *H. Crijns*[1], *D. Boff*[2], *R. Janssens*[1], *S. Struyf*[1], *F. A. Amaral*[2], *M. M. Teixeira*[2];
[1]*KU Leuven, Rega Institute, Leuven, Belgium,* [2]*UFMG, Belo Horizonte, Brazil.*

Chemokines are presented on the endothelium and immobilized in the tissue at the site of inflammation through binding to glycosaminoglycans (GAGs). This interaction ensures high local concentrations of the produced chemokines, prevents their diffusion and inhibits proteolytic degradation. Subsequently, chemokines interact with their G protein-coupled receptor(s) (GPCRs), which results in adhesion of leukocytes and extravasation through the endothelium. The binding of chemokines to GAGs has been proven indispensable for chemokine activity in vivo. We discovered that the COOH-terminal tail of CXCL9 has potent GAG-binding properties. In addition, synthetic CXCL9-derived peptides competed with active chemokines for GAG binding in an ELISA-like heparin binding assay and inhibited the in vivo recruitment of neutrophils towards the major human neutrophil attractant IL-8 or CXCL8 injected in the peritoneum or the knee cavity of mice. Reducing the length of the CXCL9 peptide, especially when one of the GAG-binding motifs was deleted, gradually decreased the capacity to compete with CXCL8 for GAG binding. Using intravital microscopy, we showed that the CXCL9 peptide coats the endothelial cell surface of blood vessels in vivo thereby inhibiting CXCL8-induced neutrophil adhesion in the murine cremaster muscle model. Intravenous application of the CXCL9-derived peptide resulted in inhibition of MSU crystal-induced neutrophil migration to the knee cavity in a mouse model of gout. In conclusion, we identified an alternative approach for inhibitors of chemokine - GPCR interactions to target the chemokine system to reduce inflammation.

WS.C6.02.06
Mechanisms by which lung surfactant protein SP-A amplifies IL-4-mediated effects on alveolar macrophages

C. Casals[1,2], *B. García-Fojedo*[2,1], *C. Montero-Fernández*[1,2], *C. Stamme*[3,4], *C. M. Minutti*[2,5];
[1]*Complutense University of Madrid, Madrid, Spain,* [2]*Biochemical Center of Respiratory Diseases (CIBERES), Madrid, Spain,* [3]*Leibniz Center for Medicine and Biosciences, Borstel, Germany,* [4]*University of Lübeck, Lübeck, Germany,* [5]*The Francis Crick Institute, London, United Kingdom.*

The phenotype of alveolar macrophages (aMϕs) is determined in part by the alveolar environment. We recently reported that aMϕs switch their phenotype by integrating IL-4 and lung-specific signals that lead to activation of tissue repair programs. Surfactant protein SP-A is a lung factor that amplifies IL-4Rα-dependent activation and proliferation of aMϕs via myosin18A receptor (Myo18A). However, the mechanism by which SP-A and IL-4 synergistically enhance activation and proliferation of aMϕs remains elusive.
Here we show that SP-A activated PI3K through binding to Myo18A receptor; accordingly, blocking PI3K activity or Myo18A receptor abrogated SP-A´s effects on IL-4 signaling. SP-A-dependent activation of PI3K and subsequent phosphorylation of its downstream effectors Akt, TSC-mTORC1, GSK3β, and PKCζ amplified IL-4-mediated aMϕ proliferation and/or alternative activation. On the one hand, SP-A sustained the PI3K-Akt-mTORC1 signaling pathway triggered by IL-4. Both alternative activation and proliferation of aMϕs induced by SP-A+IL-4 are inhibited by Akt inhibitor VIII, torin1 (mTORC1/mTORC2 inhibitor), and rapamycin (mTORC1 specific inhibitor). Our results also indicate that SP-A increased Akt-dependent phosphorylation of GSK3β, which abrogates its role in inhibiting proliferation. On the other hand, the SP-A/Myo18A/PI3K/PKCζ axis was involved in enhancing IL-4-dependent STAT6 activation and arginase activity. PKCζ inhibition by PKCζ pseudosubstrate was able to reduce IL-4+SP-A-driven p-STAT6 and alternative activation, but not proliferation.
In conclusion, SP-A activates PI3K-dependent coordinated signaling pathways that amplify IL-4 actions in cell proliferation and acquisition of effector functions.
This study was supported by Spanish Ministry of Economy and Competitiveness (SAF2015-65307-R) and Institute of Health Carlos III (CIBERES CB06/06/0002).

WS.C6.03 Immune cells in tissue fibrosis

WS.C6.03.01
Plasmacytoid dendritic cells promote lung and skin fibrosis

R. R. Singh, *S. Kafaja, A. Divekar, R. Saggar, F. Abtin, D. Khanna, D. Furst, I. Valera;*
David Geffen School of Medicine, UCLA, Los Angeles, United States.

Fibrosis is the end-result of most inflammatory conditions, but its pathogenesis remains unclear. Here, we investigated the role of plasmacytoid dendritic cells (pDC) in the pathogenesis of systemic fibrosis using the bleomycin-induced skin and lung fibrosis model and clinical samples from patients with systemic sclerosis (SSc). We demonstrate that in animals and humans with systemic fibrosis, pDCs are unaffected or reduced systemically (spleen/peripheral blood) but they increase in the affected organs (lungs/skin/bronchoalveolar lavage). Depletion of pDCs in animals ameliorated bleomycin-induced lung and skin lung fibrosis, and reduced infiltration with B-cells, T-cells, and natural killer T-cells in the affected organs but not in spleen. pDC-depleted bleomycin-injected mice also had a reduced expression of genes and proteins involved in chemotaxis, dendritic cell differentiation, inflammation, and fibrosis in the lungs as compared to controls. In resonance with animal findings, the frequency of pDCs in the lungs of patients with SSc correlated with the severity of lung disease, and with the frequency of $CD4^+$ and $IL\text{-}4^+$ T-cells in the lung. Finally, treatment with a tyrosine kinase inhibitor imatinib that has been reported to reduce and/or prevent deterioration of skin and lung fibrosis profoundly reduced pDCs in lungs but not in peripheral blood of patients with systemic sclerosis. These observations suggest a role of pDCs in the pathogenesis of systemic fibrosis and identify the increased trafficking of pDCs to the affected organs as a potential therapeutic target in fibrotic diseases.

WS.C6.03.02
S100A8/A9 promotes parenchymal damage and renal fibrosis in obstructive nephropathy

***A. Tammaro**;*
AMC, Amsterdam, Netherlands.

Despite advances in our understanding of the mechanisms underlying progression of chronic kidney disease and the development of fibrosis, only limited efficacious therapies exist. The calcium binding protein S100A8/A9, is a damage-associated molecular pattern which can activate TLR4 or RAGE. Activation of these receptors is involved in the progression of renal fibrosis, however the role of S100A8/A9 herein remains unknown. Therefore, we analysed S100A8/A9 expression in patients and mice with obstructive nephropathy and subjected wild-type and S100A9 KO mice, lacking the heterodimer S100A8/A9, to Unilateral Ureteral Obstruction (UUO). We found profound S100A8/A9 expression in granulocytes that infiltrated human and murine kidney, and enhanced renal expression over time following UUO. S100A9 KO mice were protected from UUO-induced renal fibrosis, independently of leukocyte infiltration and inflammation.
Loss of S100A8/A9 protected tubular epithelial cells from UUO-induced apoptosis and critical epithelial-mesenchymal transition steps. In vitro studies revealed S100A8/A9 as a novel mediator of epithelial cell injury, through loss of cell polarity, cell cycle arrest and subsequent cell death. In conclusion, we demonstrate that S100A8/A9 mediates renal damage and fibrosis presumably through loss of tubular epithelial cell contacts and irreversible damage. Suppression of S100A8/A9 could be a therapeutic strategy to halt renal fibrosis in patients with chronic kidney disease.

WS.C6.03.03
CXCL4 drives inflammation and fibrosis on monocyte-derived dendritic cells through transcriptional and epigenetic imprinting

***S. C. Silva-Cardoso**[1], W. Tao[1], C. Angiolilli[1], A. Lopes[1], C. Bekker[1], J. van Laar[1], E. Hack[1], R. de Boer[2], M. Boes[1], A. Pandit[1], T. Radstake[1];*
[1]University Medical Center, Utrecht, Utrecht, Netherlands, [2]Theoretical Biology group, University Utrecht, Utrecht, Netherlands.

Accumulation of extracellular matrix (ECM) or fibrosis is one of the hallmarks that characterizes the pathogenesis of systemic sclerosis (SSc), together with immune dysregulation and small vessel vasculopathy. CXCL4 (Chemokine CXC motif ligand 4) levels are increased in SSc patients and correlated with skin and lung fibrosis. We and others shown that CXCL4 modulates phenotype and function of immune cells, however how CXCL4 modulates immune cell responses remains unclear. Here we investigated the impact of CXCL4 exposure on the transcriptome and DNA methylation during monocyte-derived dendritic cells (moDCs) differentiation and stimulation with polyI:C. Integration of high-throughput data reveals that CXCL4 drives to dramatic changes on the transcriptome and DNA methylation. This is reflected in the dysregulation of metabolic pathways, HIF-1 signaling, innate and adaptive immune pathways. Also, CXCL4 potentiates a novel function to moDCs, namely the production of ECM molecules, such as fibronectin (FN1) and TGFβ1. CXCL4 exposure results in epigenetic imprinting during moDC differentiation and gene regulatory network analysis reveal that CXCL4 dysregulates key transcriptional regulators. In conclusion, we show that CXCL4 besides driving to dramatic changes on moDC phenotype and function, this chemokine is the first endogenous ligand that leads to innate immune training. Interestingly, we found for the first time the direct implication of CXCL4 on the production of ECM by moDCs, thereby underscoring the role of CXCL4 in inflammatory and fibrotic conditions such as SSc. Supported by: Portuguese FCT No.SFRH/BD/89643/2012 (SCSC); China Scholarship Council (CSC) No.201606300050 (WT); ERC starting grant (CIRCUMVENT) and Arthritis foundation grant (TRDJR)

WS.C6.03.04
Liver sinusoidal endothelial cells trigger CD8 T cell mediated liver failure and herald liver regeneration

***M. Luedemann**[1], A. Goroi[2], L. A. D'Alessandro[2], S. Donakonda[1], U. Klingmueller[2], P. A. Knolle[1];*
[1]Institute of Molecular Immunology and Experimental Oncology, Technical University of Munich, Munich, Germany, [2]German Cancer Research Center, Heidelberg, Germany.

Introduction: Liver-sinusoidal-endothelial-cells (LSECs) have unique immune features that enable them to coordinate local hepatic immune responses. Recently, we showed that LSECs trigger fulminant viral hepatitis through antigen cross-presentation to CD8 T cells leading to perforin-dependent LSEC-killing. This results in perfusion-deficits causing liver-failure. Here, we address whether LSECs also contribute to liver regeneration by HGF-expression. **Material and Methods:** Primary cell cultures of LSEC were established to investigate HGF-expression, proteomics/secretomics of *in vitro* stimulated LSECs for determination of their pro-regenerative potential, *in vitro* LSEC cross-presentation to T cells and its contribution to HGF production. **Results:** Exposing LSECs to dying hepatocytes, extracellular ATP, or other P2X7 receptor-ligands failed to induce HGF-production excluding a sentinel role for local cell death promoting liver regeneration. However, LSECs produced HGF after IL-6 cluster-signalling in a dose-dependent fashion. Since IL-6 cluster-signalling is induced in LSECs upon mutual activation during cross-presentation to CD8 T cells, HGF levels were also increased after LSEC-activation of CD8 T cells. LSEC Proteome/secretome analysis revealed that upon IL-6 cluster-signalling pro-HGF levels decreased and levels of matured HGF increased, consistent with increased enzymatic activity upon IL-6 cluster-signalling of e.g. urokinase, to convert inactive pro-HGF into bioactive matured HGF. **Conclusion:** We discovered a pivotal role for LSEC in triggering T cell-mediated liver damage but also promoting liver regeneration through production of bioactive HGF. Generation of bioactive HGF by LSECs in response to local T cell activation may act in a pre-emptive fashion to kickstart liver regeneration already at the time of impending immune-mediated liver damage.

WS.C6.03.05
NLRP3- A key in the mediation of colitis into colitis-associated colorectal cancer

***A. P. Perera**, R. Eri;*
Shool of Health Sciences, Launceston, Australia.

Ulcerative colitis is a known risk for development of colorectal cancer but the exact mechanism of how chronic inflammation induce cancer has not been established. Many studies have indicated a role for NLRP3 inflammasome in colitis and tumorigenesis but the results have been controversial due to different chemical models of colitis induction and altered microbiota. To address the inconclusive role of NLRP3 we have designed the first murine model deficient in NLRP3 in a spontaneous chronic colitis mouse model Winnie (*Muc2* mutant). Extensive studies done in Winnie has proven it to be arguably the best available murine model to study ulcerative colitis and its pathogenesis. Our results show colon tumorigenesis in Winnie x NLRP3$^{-/-}$ with high burden of colonic polyps starting at 12 weeks. NLRP3$^{-/-}$x Winnie mice have significantly shorter colons, and a higher ratio of colon weight to length indicating the severity of colitis. Histological examination NLRP3$^{-/-}$x Winnie colon revealed differentiated adenocarcinoma with high-grade dysplasia and hyperplasia regions. Extracted RNA from colonic segments was used for analysis of colorectal cancer biomarker gene expression using PCR micro array. Colon organ culture supernatants were assayed via Bio-Plex for proinflammatory cytokines. Flow cytometry was done to immunophenotype T cells, B cells, neutrophils and natural killer cells in *WinniexNlrp3*$^{-/-}$. We analysed NLRP3$^{-/-}$x Winnie faecal metabolomics and characterized the faecal microbiota with 16S rRNA to identify specific microbial signature of colitis associated colon cancer. Our results describes the role of NLRP3 inflammasome in colorectal cancer leading to the development of novel therapeutic tools.

WS.C6.03.06
The IgE-FcεR1 axis strongly promotes epithelial hyperplasia and inflammation-driven skin carcinogenesis

***M. Hayes**, S. Ward, L. Wang, R. Castro Seoane, G. Crawford, J. Strid;*
Imperial College, London, United Kingdom.

The skin is a potent site for induction of type 2 immunity and IgE. Previous work has shown that accumulation of IgE effector cells in the skin is a consistent hallmark of skin perturbation and inflammation. Little is however known about the physiological role of IgE-carrying cells in the skin. Here, we examine the role of IgE and IgE effector cells in skin inflammation and epithelial carcinogenesis driven by chronic inflammation. We show that the inflammation-driven outgrowth of skin tumours is highly dependent on IgE and FcεRI^{+} effector cells. Mice lacking IgE or FcεRI developed significantly fewer and smaller tumours in a two-stage chemical carcinogenesis model. During the 'tumour-promoting' skin inflammation, high numbers of IgE-carrying basophils and mast cells accumulated in the skin whereas basophils selectively also infiltrated the tumours. Skin basophils were more immune active than mast cells and expressed high levels of IL-4, IL-6, IL-13, histamine and prostaglandins. The production of type 2 cytokines, as well as release of histamine was critically dependent on IgE and FcεRI. Further *in vivo* and *in vitro* analysis demonstrated that basophils, in an IgE-dependent manner, induced keratinocyte activation, proliferation and differentiation. Similar results were found when primary keratinocytes were cultured with histamine alone, suggesting that the keratinocyte response was mainly induced via engagement of histamine receptors. Collectively, this work demonstrate that IgE effector cells, predominantly basophils, exacerbate skin inflammation, induce epidermal hyperplasia and play a key role in promoting inflammation-driven carcinogenesis.

WS.D1.01 Mucosal immune regulation

WS.D1.01.01
Transcription factor c-Maf is required for the control of Th17 cell responses by intestinal regulatory T cells

***H. Hussein**, S. Denanglaire, Y. Ajouaou, O. Leo, F. Andris;*
Laboratory of Immunobiology, Gosselies, Belgium.

Intestinal Tregs selectively inhibit immune responses directed against commensal microorganisms and allow responses against pathogens, thereby maintaining intestinal homeostasis and avoiding chronic inflammation. Recent observations suggest that tissue-resident Tregs are a specialized subset of Tregs whose phenotype and function are strongly influenced by their environment. In contrast to most splenic Tregs, intestinal Tregs express high levels of c-Maf, a bZIP transcription factor involved in the differentiation and function of multiple helper T cell subsets.
Ablation of c-Maf in Tregs results in the development of spontaneous colitis, characterized by a rectal prolapse, increased Th17-associated cytokine expression and expansion of Th17 cells in the intestine. The increased Treg percentage suggests that the spontaneous colitis is a consequence of an altered Treg function rather than a quantitative defect. RORγt$^+$ Tregs are induced in the intestine by the microbiota. Their colocalization with Th17 cells and high IL-10 expression levels makes them particularly apt at controlling Th17 responses. Our results show the loss of RORγt$^+$ Tregs in the intestine of c-Maf deficient mice, suggesting a selective role for c-Maf in the differentiation of this subset. Current investigations aim to decipher the signaling pathways leading to c-Maf expression in gut-associated Tregs and identify genes controlled by c-Maf. We hope that our study will contribute to a better understanding of tissue resident Treg origin and function both in health and disease.
This work is supported by a Fund for Research Training in Industry and Agriculture fellowship from the Fund for Scientific Research.

WS.D1.01.02
Induction of intestinal Th22 cells by segmented filamentous bacteria

***U. Roy**[1], E. Galvez[1], A. Gronow[1], M. Basic[2], A. Bleich[2], S. Huber[3], T. Strowig[1];*
[1]Helmholtz Centre for Infection Research, Braunschweig, Germany, [2]Medical University Hannover, Hannover, Germany, [3]University Hospital Hamburg-Eppendorf, Hamburg, Germany.

Heterogeneous effector CD4+ T cells play an important role in modulating inflammation via their ability to produce distinct cytokines. Recently, Th22 cells have been identified as an important contributor of protection against enteropathogenic infection. While commensals inducing intestinal Th1, Th17 and Treg cells have been identified and extensively studied, microbiota-induced Th22 cells have not been reported yet.
To assess the influence of microbiota composition to shape anti-bacterial IL-22 production, we utilized cytokine knock-in reporter mice (IL-22BFP x IL-17AGFP) colonized with different SPF and non-SPF communities as well as colonization with segmented filamentous bacteria (SFB). Early after enteric *Salmonella* Typhimurium (*S.* Tm) infection CD4+ T cells are detected that are able to secrete *in vivo* IL-22, alone or together with IL-17A and we show that their abundance is modulated by microbiota composition. We demonstrate that SFB colonization is sufficient to induce Th22 cells and that they share a similar TCR V_β14 enrichment than Th17 cells. Comparison of gene expression in IL-17A and IL-22 producing CD4+ T cells revealed significant differences between Th17 cells present in the steady state in the terminal ileum as well as Th22 and Th17 cells present during *S.* Tm infection, such as the expression of IFN_γ and IL-17F. In addition, we provide evidence that Th22 cells develop independent of IL-17 production using IL-17 fate mapping mice.
Together, our study identifies that SFB induces Th22 cells and that they differ from Th17 cells in their development and during infections in their gene expression profiles.

WS.D1.01.03
Microbiota sensing by a Mincle-Syk axis in dendritic cells promotes intestinal immune barrier via the steady-state regulation of IL-17 and IL-22

*M. Martínez-López[1], S. Iborra[1], A. Mastrangelo[1], R. Conde-Garrosa[1], C. Danne[2], D. M. Reid[3], G. D. Brown[3], D. Bernardo[4], S. Leibundgut-Landmann[5], **D. Sancho**[1];*
[1]CNIC, Madrid, Spain, [2]Kennedy Institute for Rheumatology, Oxford, United Kingdom, [3]University of Aberdeen, Aberdeen, United Kingdom, [4]Hospital Universitario de la Princesa, Madrid, Spain, [5]University of Zurich, Zurich, Switzerland.

Maintenance of the intestinal barrier function keeps homeostatic host-microbiota relationship and depends on the cytokines IL-17 and IL-22. Production of these cytokines by type 17 T-helper (Th17) cells and group 3 innate lymphoid cells (ILC3) is influenced by the gut microbiota in steady state. However, the host pathways that sense commensal microbiota to trigger these immune responses need further exploring. Here, we show that a Syk kinase-coupled signaling pathway in dendritic cells (DCs) is critical for commensal-dependent production of intestinal IL-17 and IL-22. We identify Mincle as a Syk-coupled receptor that detects mucosal-resident commensals and triggers IL-6 and IL-23p19 expression, cytokines that regulate intestinal Th17 and ILC3 function. Absence of Mincle or Syk in DCs impairs antimicrobial peptide and IgA production at the intestinal epithelium. Thus, sensing of commensals by Mincle and Syk in DCs reinforces intestinal immune barrier and limits systemic microbial translocation, promoting host-microbiota mutualism.

WS.D1.01.04
Control of RORγt$^+$ regulatory T cell differentiation and intestinal immune homeostasis is dependent on the transcription factor c-Maf

***C. Neumann**[1,2], J. Blume[3,4], A. Vasanthakumar[3,4], A. Beller[1], U. Roy[5], Y. Liao[6,7], F. Heinrich[1], C. Stehle[1], G. A. Heinz[1], P. Maschmeyer[1], T. Sidwell[3,4], Y. Hu[6,7], D. Amsen[8], C. Romagnani[1], H. Chang[1], A. Kruglov[1], M. Mashreghi[1], W. Shi[6,7], T. Strowig[5], S. Rutz[9], A. Kallies[3,4], A. Scheffold[1,2];*
[1]German Rheumatism Research Centre (DRFZ), Leibniz Association, Berlin, Germany, [2]Department of Cellular Immunology, Clinic for Rheumatology and Clinical Immunology, Charité - Universitätsmedizin, Berlin, Germany, [3]Molecular Immunology Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [4]Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia, [5]Microbial Immune Regulation Research Group, Helmholtz Centre for Infection Research, Braunschweig, Germany, [6]Department of Medical Biology, University of Melbourne, Melbourne, Australia, [7]Bioinformatics Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [8]Department of Hematopoiesis, Sanquin Landsteiner Laboratory for Blood Cell Research, Amsterdam, Netherlands, [9]Department of Cancer Immunology, Genentech, San Francisco, United States.

Foxp3$^+$ regulatory T (Treg) cells are essential for immunological tolerance and homeostasis. In peripheral tissues, Treg cells acquire enhanced suppressive functions and co-opt distinct transcriptional modules, allowing context and tissue-dependent immune regulation. Here we show that the transcription factor c-Maf was highly expressed by effector Treg cells and controlled their IL-10 production. In the intestine, c-Maf was required for the differentiation of RORγt$^+$ microbiota-dependent Treg cells, and restricted their production of inflammatory cytokines. Molecular profiling revealed that c-Maf regulated expression of key genes of the transcriptional signature of intestinal Treg cells, including *Rorc* and *Il10*. Consequently, Treg cell-specific loss of c-Maf resulted in a selective deregulation of intestinal Th17 responses, which protected mice from acute colon inflammation. In addition, Treg cell-specific c-Maf-deficiency reduced the diversity and altered the composition of the intestinal microbiota. Importantly, colonization of wild-type germ-free mice with reprogrammed microbiota from Treg-specific c-Maf-deficient mice similarly skewed the balance of RORγt$^+$ Treg/Th17 cells and increased colitis resistance, demonstrating that c-Maf-dependent Treg cells controlled host-microbial symbiosis through an interdependent regulatory loop. Thus, our study identifies a key role of c-Maf in preserving the identity and function of intestinal Treg cells, essential for the control of intestinal immune homeostasis and inflammation.

WS.D1.01.05
IL-17 receptor signaling in intestinal secretory progenitor cells regulates epithelial cells regeneration

***P. Kumar**, M. Beaupre, K. Chang, A. Banerjee, T. Chu, H. Huang, X. Lin, S. Khalid, P. Joshi, A. Bialowska, V. Yang;*
Stony Brook University, Stony Brook, United States.

Introduction IL-17A, derived from Th17 cells, plays an important role in intestinal host defense. However, the interaction and potential molecular synergies between IL-17A and epithelial cell lineages, intestinal stem cells (ISCs), and progenitors of the intestine remain unclear. Materials and Methods *Il17ra$^{fl/fl}$;Villin-cre* and *Il17ra$^{fl/fl}$;Atoh1-cre* mice were generated. A Dextran Sulfate Sodium (DSS)-mediated epithelial injury model was used. RT-PCR, immunofluorescence, and primary organoid cultures were used to study impact of IL-17A signaling on specific cell types. Results Our data show that knockout of IL-17RA from the entire intestinal epithelia in *Il17ra$^{fl/fl}$;Villin-cre* mice are more susceptible to DSS-induced colitis. We found a defect in secretory goblet cell number in DSS-administered *Il17ra$^{fl/fl}$;villin-cre* mice. Atonal homolog 1 (Atoh1) is a transcription factor required for intestinal secretory including goblet cell differentiation. Our preliminary data show a more severe DSS-induced colitis in *Il17ra$^{fl/fl}$;Atoh1-cre* mice, suggesting secretory progenitor or mature secretory epithelium-specific IL-17RA signaling regulates epithelial cell regeneration. UEA-1 and alcian blue stained colon tissues revealed reduced goblet cell number in *Il17ra$^{fl/fl}$;Atoh1-cre$^+$* mice. Next, we found reduced Sox9 expression as well as reduced Ki67$^+$ stained cells in the colon of *Il17ra$^{fl/fl}$;Atoh1-cre$^+$* mice. Furthermore, we show that IL-17A does not regulate mature goblet cell-specific functions. These data indicate a stem cell-specific defect. Based on this observation, organoids from *Il17ra$^{fl/fl}$;Atoh1-cre* mice were continuously stimulated with IL-17A. Continuous IL-17A stimulation lead to reduced organoid budding/morphogenesis, further confirming a IL-17A-mediated role in Atoh1-dependent epithelial cell regeneration. Conclusion Our data show a novel role of IL-17A in regulating Atoh1-dependent epithelial cell regeneration.

WS.D1.01.06
Anti-commensal IgG augments intestinal inflammation in ulcerative colitis via IL-1 beta-dependent type 17 immunity

T. Castro-Dopico[1], *T. W. Dennison*[1], *J. R. Ferdinand*[1], *R. Mathews*[1], *D. Clift*[2], *B. Stewart*[1], *C. Jing*[1], *K. Strongili*[3], *E. Monk*[1], *K. Saeb-Parsy*[4], *C. E. Bryant*[5], *M. Parkes*[3], *M. Clatworthy*[1];
[1]*Molecular Immunity Unit, Department of Medicine, University of Cambridge, Cambridge, United Kingdom,* [2]*MRC Laboratory of Molecular Biology, Cambridge, United Kingdom,* [3]*Division of Gastroenterology, Cambridge Universities NHS Foundation Trust, Cambridge, United Kingdom,* [4]*Department of Surgery, University of Cambridge, Cambridge, United Kingdom,* [5]*Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom.*

Inflammatory bowel disease (IBD) is a chronic relapsing condition with two major subtypes, Crohn's disease (CD) and ulcerative colitis (UC), both driven by aberrant immune responses to commensals. One of the strongest genetic associations in UC is with *FCGR2A*, the gene encoding an activating Fcγ receptor. FcγRs mediate the cellular effector functions of IgG. The UC-associated FcγRIIA-R131 variant is protective and reduces affinity for IgG. Since IgA is the dominant mucosal antibody, the role of IgG and FcγRs in IBD pathogenesis has been largely overlooked. Here we sought to investigate anti-commensal IgG responses in patients with UC and to determine the mechanism by which local IgG-FcγR engagement might contribute to intestinal inflammation in UC and murine models of dextran sodium sulfate (DSS)-induced colitis. We observed a significant increase in IgG-bound commensals and activated FcγR signalling pathways in UC patients and DSS-inflamed colon. The genes most robustly correlated with *FCGR2A* expression were *IL1B* and *CXCL8*, with hierarchical clustering confirming *IL1B* as the gene most closely associated with *FCGR2A*. Intestinal macrophages were the principle source of IL-1β and *ex vivo* stimulation with IgG immune complexes induced ROS-dependent IL-1β production mediated by activation of the NLRP3 inflammasome. *In vivo* manipulation of the macrophage FcγR activating/inhibitory ratio in mice determined IL-1β and Th17 cell induction. Finally, IL-1β blockade in mice with a high FcγR A/I ratio reduced IL-17 and IL-22-producing T cells and the severity of colitis. Our data reveal that commensal-specific IgG contributes to intestinal inflammation via FcγR-dependent, IL-1β-mediated Th17 activation.

WS.D1.02 Innate responses and immune signaling

WS.D1.02.01
The microbiota protects against respiratory infection via GM-CSF signaling

T. B. Clarke*, R. L. Brown, R. P. Sequeira;*
Imperial College London, London, United Kingdom.

The microbiota promotes resistance to respiratory infection, but the mechanistic basis for this is poorly defined. Here, we identify members of the microbiota that protect against respiratory infection by the major human pathogens *Streptococcus pneumoniae* and *Klebsiella pneumoniae*. We show that the microbiota enhances respiratory defenses via granulocyte-macrophage colony-stimulating factor (GM-CSF) signaling, which stimulates pathogen killing and clearance by alveolar macrophages through extracellular signal regulated kinase signaling. Increased pulmonary GM-CSF production in response to infection is primed by the microbiota through interleukin-17A. By combining models of commensal colonization in antibiotic-treated and germ-free mice, using cultured commensals from the Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria phyla, we found that potent Nod-like receptor-stimulating bacteria in the upper airway (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and intestinal microbiota (*Lactobacillus reuteri, Enterococcus faecalis*, *Lactobacillus crispatus* and *Clostridium orbiscindens*) promote resistance to lung infection through Nod2 and GM-CSF. Our data reveal the identity, location, and properties of bacteria within the microbiota that regulate lung immunity, and delineate the host signaling axis they activate to protect against respiratory infection.

WS.D1.02.02
Hypercholesterolemia affects macrophage metabolism and function

J. Baardman[1], *M. van Weeghel*[1], *S. Verberk*[2], *K. H. Prange*[1], *M. P. de Winther*[1], ***J. Van den Bossche***[2];
[1]*Academic Medical Center, Amsterdam, Netherlands,* [2]*VU University Medical Center, Amsterdam, Netherlands.*

Metabolic reprogramming has emerged a crucial regulator of immune cell activation but how systemic metabolism influences immune cell metabolism and function remain to be investigated. Here we demonstrate that blood leukocytes from familial hypercholesterolemia (FH) patients show reduced expression of genes related to oxidative phosphorylation. To investigate the effect of dyslipidemia on immune cell metabolism, we performed in-depth transcriptional, metabolic and functional characterization of macrophages isolated from hypercholesterolemic mice. Systemicmetabolic changes in such mice translate into altered cellular macrophage metabolism and attenuates inflammatory macrophage responses. Apart from diminished maximal mitochondrial respiration, LPS-mediated induction of the pentose phosphate pathway (PPP) was reduced. Our observation that suppression of the PPP diminished LPS-induced cytokine secretion supports the notion that this pathway is a key feature of inflammatory macrophage responses. Overall, this study reveals that systemic and cellular metabolism are strongly interconnected and together dictate macrophage phenotype and function.

WS.D1.02.03
MR1 recognition by human γδ T cells

J. Le Nours[1,2], *N. Gherardin*[3,4], *S. Ramarathinam*[1], *W. Awad*[1,2], *B. Gully*[1,2], *R. Berry*[1,2], *F. Wiede*[1], *D. Fairlie*[5,6], *T. Tiganis*[1], *J. McCluskey*[3], *D. Pellicci*[3,4], *A. Uldrich*[3,4], *A. Purcell*[1], *D. Godfrey*[3,4], *J. Rossjohn*[1,2,7];
[1]*Biomedicine discovery institute, Monash university, Clayton, Australia,* [2]*Australian Research Council Centre of Excellence in Advanced Molecular Imaging, Monash University, Clayton, Australia,* [3]*Peter Doherty Institute for Infection and Immunity, University of Melbourne, Parkville, Australia,* [4]*Australian Research Council Centre of Excellence in Advanced Molecular Imaging, University of Melbourne, Parkville, Australia,* [5]*Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia,* [6]*Australian Research Council Centre of Excellence in Advanced Molecular Imaging, University of Queensland, Brisbane, Australia,* [7]*Institute of Infection and Immunity, Cardiff University School of Medicine, Cardiff, United Kingdom.*

The T lymphocytes repertoire is divided into two major lineages, αβ and γδ T cells, that are defined by their T cell receptor (TCR) gene-segment usage. The MHC-like molecule MR1 presents Vitamin-B derivatives to mucosal-associated invariant T cells (MAIT). Using MR1 tetramers, we characterized a population of MR1-restricted human γδ T cells that included phenotypically diverse Vγ8-Vδ1, Vγ9-Vδ1 and Vγ8-Vδ3 subsets, all of which exhibited MR1 autoreactivity, independent on the nature of the bound ligand. The crystal structure of a γδTCR-MR1-antigen complex showed the γδTCR docked in a highly unusual manner that starkly contrasted all other TCR complex structures. The γδTCR bound under the MR1 antigen-binding cleft. Contacts were mediated largely by the TCR δ-chain and more surprisingly by the α3-domain of MR1. Our findings reshape our understanding of TCR recognition determinants and γδ T cells.

WS.D1.02.04
RNA helicase DDX3 is a potent inducer of type I interferon and adaptive immunity

M. Stunnenberg*, S. I. Gringhuis, T. B. Geijtenbeek;*
Academic Medical Center, Amsterdam, Netherlands.

Strong innate and adaptive immune responses are paramount to prevent infection. We have recently identified the RNA helicase human DEAD-box polypeptide 3 (DDX3) as a powerful pattern recognition receptor (PRR) to HIV-1 in dendritic cells (DCs). Here, we have investigated the capacity of DDX3 to induce potent innate and adaptive immune responses upon triggering with synthetic HIV-1-derived RNA ligands. DDX3 recognizes abortive HIV-1 RNA that is generated during HIV-1 transcription and encodes for the first 58-nucleotides of trans-activating protein Tat. Stimulation of DDX3 in DCs with synthetic abortive HIV-1 RNA induced IFNβ as well as IFN-stimulated genes such as TRIM5α and MxA. The type I IFN responses were functional as they strongly inhibited the replication capacity of HIV-1 in DCs. We next investigated whether DDX3 triggering with abortive RNAs also induce adaptive immune responses. DC treatment with abortive RNAs induced DC maturation and pro-inflammatory cytokine responses. Moreover, DCs stimulated with abortive RNAs that were co-cultured with peripheral blood lymphocytes enhanced CD4+ and CD8+ T cell proliferation, thus supporting a role for DDX3 in inducing adaptive immunity. Comparison between putative viral ligands for DDX3 and other RIG-I-like receptors (RLRs) identified the abortive RNAs as the most potent triggers of immune responses. Abortive RNAs can be used as novel adjuvants for vaccine design and immunotherapy studies, since subsequent triggering of DDX3 evokes strong and functional antiviral immune responses. Thus, we have identified DDX3 as pattern recognition receptor of the RIG-I-like family by sensing HIV-1 and other viruses. Aidsfonds grant number P-9906.

WS.D1.02.05
Recruitment of DC-SIGN$^+$ monocyte-derived dendritic cells in the skin for antigen delivery and adaptive immunity

S. T. Schetters*, L. J. Kruijssen, M. H. Crommentuijn, J. den Haan, Y. van Kooyk;*
VU University Medical Center, Amsterdam, Netherlands.

Delivery of antigenic compounds to dendritic cells remains key to vaccination efficacy against tumors and pathogens. We have investigated the C-type lectin, DC-SIGN, as a target on dendritic cells to efficiently deliver antigens for the initiation of protective adaptive immune responses. Here, we show that subcutaneous injection of the adjuvant MF59 attracts mDC-SIGN$^+$ antigen presenting cells to the skin for antigen delivery. We identify the time-dependent influx of neutrophils and monocytes, including MoDCs, to the skin. Next, targeting mDC-SIGN$^+$ skin-infiltrating cells with ovalbumin-coupled anti-mDC-SIGN antibody with adjuvant induces multifunctional antigen-specific CD8$^+$ T cells, as well as antigen-specific antibody responses. Interestingly, when the vaccine is administered directly with the adjuvant, cytotoxic CD8$^+$ T cell responses are preferred, whereas antibody production is boosted when the vaccine is delivered 24 hours after adjuvant vaccine site preconditioning. Hence, we demonstrate a unique approach to facilitate optimal antigen delivery and adaptive immunity shaped by a time-dependent sequence of vaccination events.

WS.D1.02.06

Enhanced trained immunity by targeting SHIP-1 in myeloid cells

P. Saz-Leal[1], *C. del Fresno*[1], *P. Brandi*[1], *S. Martínez-Cano*[1], *O. M. Dungan*[2], *J. D. Chisholm*[2], *W. G. Kerr*[2,3,4], *D. Sancho*[1];
[1]*Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain,* [2]*Department of Chemistry, Syracuse University, Syracuse, NY 13210, United States,* [3]*Department of Microbiology and Immunology, State University of New York (SUNY) Upstate Medical University, Syracuse, NY 13210, United States,* [4]*Pediatrics Department, SUNY Upstate Medical University, Syracuse, NY 13210, United States.*

Introduction: β-glucan-induced trained immunity in myeloid cells leads to long-term protection against secondary infections through activation of the Dectin-1/PI3K (Phosphoinositide 3-kinase) pathway. While previous studies have addressed the characterization of this phenomenon, strategies to boost trained immunity deserve further investigation. SHIP-1 is a hematopoietic-restricted inositol poly-phosphatase that limits PI3K activity and associates with Dectin-1. Thus, we wondered whether targeting SHIP-1 could modulate Dectin-1-mediated training.
Methods: Bone marrow-derived macrophages (BMDMs) from WT and LysM-*Cre*-SHIP-1 (LysMΔ*SHIP-1*) mice were subjected to a β-glucan-induced trained immunity *in vitro* model. Receptor expression, cytokine production, pathway activation and metabolic status were analyzed. *In vivo,* both WT and LysMΔSHIP-1 mice were trained with β-glucan or *Candida albicans* and either challenged with LPS to evaluate cytokine production or lethally infected with *C. albicans* to monitor protection. We also tested the effect of the SHIP-1 inhibitor 3AC (SHIPi) in our *in vitro* and *in vivo* models and in human peripheral blood mononuclear cells (PBMCs).
Results: β-glucan-trained SHIP-1-deficient macrophages enhanced TNFα production to a secondary LPS challenge, correlating with increased phosphorylation of Akt, targets of mTOR and elevated glycolytic metabolism. This enhanced training in SHIP-1-deficient macrophages relied on epigenetic modifications. Trained LysMΔ*SHIP-1* mice showed increased TNFα production upon LPS challenge *in vivo* and better protection against reinfection with *C. albicans*. Pharmacological inhibition of SHIP-1 enhanced trained immunity against *Candida* infection, in mouse macrophages and human peripheral blood mononuclear cells.
Conclusions: Our data establish a proof of concept for trained immunity improvement and a strategy to achieve it by targeting SHIP-1.

WS.D1.03 Regulation of effector Immune responses

WS.D1.03.01

Intestinal dysbiosis driven by dietary trp deprivation limits myelin-reactive T cell responses in a murine multiple sclerosis model

J. K. Sonner[1], *M. Keil*[1], *M. Falk-Paulsen*[2], *R. Bharti*[2], *N. Mishra*[2], *M. Kramer*[1], *K. Deumelandt*[1], *L. Wolf*[1], *I. Oezen*[1], *T. V. Lanz*[1], *I. Brandao*[3], *F. Wanke*[4], *Y. Tang*[4], *J. G. Okun*[5], *F. Kurschus*[4], *C. Reinhardt*[3], *W. Wick*[5,1], *P. Rosenstiel*[2], *M. Platten*[6,1];
[1]*German Cancer Research Center, Heidelberg, Germany,* [2]*Institute of Clinical Molecular Biology, Kiel, Germany,* [3]*University Hospital Mainz, Mainz, Germany,* [4]*Institute of Molecular Medicine, Mainz, Germany,* [5]*University Hospital Heidelberg, Heidelberg, Germany,* [6]*University Hospital Mannheim, Mannheim, Germany.*

Multiple sclerosis (MS), the most common neurological disorder among young adults, is thought to be mainly driven by auto-reactive T cells that infiltrate the central nervous system (CNS). Recent data from preclinical studies suggest that dietary components profoundly affect self-reactive T cell responses even in remote organs such as the CNS. The essential amino acid tryptophan (trp) and its metabolites have been identified as important modulators of local and systemic immune responses. While previous studies focused on trp as a source for endogenous aryl hydrocarbon receptor (AHR) ligands, we addressed the question whether dietary trp deprivation itself regulates myelin-reactive T cell responses using the murine experimental autoimmune encephalomyelitis (EAE) model.
In this study we demonstrate that omission of protein, but also selectively trp from the diet protected from CNS autoimmunity. Dietary trp depletion did not prevent priming of myelin oligodendrocyte glycoprotein-reactive T cells, yet, attenuated encephalitogenicity of circulating T cells and prevented their infiltration into the CNS. Protection from EAE induction was independent of the host's ability to sense trp via the stress kinase general control non-derepressible 2 (GCN2), but was critically dependent on the presence of the gut microbiome. Dietary trp deprivation caused mild, but evident intestinal inflammation, and, as revealed by 16S rDNA sequencing, induced substantial dysbiosis of the gut microbiome composition.
In summary, our data provide an important insight into the regulation of autoimmunity by dietary constituents and subsequent perturbations of gut microbiome homeostasis.

WS.D1.03.02

Gut dysbiosis induces dendritic-cell traffic from colon to pancreatic lymph node: implications for activation of islet-reactive T cells in type 1 diabetes

R. Toivonen[1], *S. Silojärvi*[1], *S. Pöysti*[1], *E. Yatkin*[2], *A. Takeda*[1], *M. Miyasaka*[3,1], *A. Hänninen*[1,4];
[1]*University of Turku, Turku, Finland,* [2]*University of Turku Central Animal Laboratory, Turku, Finland,* [3]*Osaka University, Osaka, Japan,* [4]*Turku University Central Hospital, Turku, Finland.*

Studies in humans and mice suggest an important role for gut microbiota in the development of autoimmune type 1 diabetes (T1D). However, the exact mechanisms by which microbiota and gut immune system influence the development of T1D remain elusive. Using KikGR-reporter mice, we investigated the possible connection between the gut and pancreatic lymph nodes (PaLN) and the effects of dysbiosis on the traffic and activation of dendritic cells (DC) and T cells. We observed significant migration of dendritic cells (DC) from colon into colon-draining mesenteric lymph nodes (coMLN) but not to PaLN. A mild infection with *Citrobacter rodentium* allowed the same DC populations to appear also in PaLN, indicating that dysbiosis activates a route from colon to PaLN. *C. rodentium* also upregulated IFNγ production by T cells. Migrated DC expressed XCR1, CD11b and CD103, typical for gut-derived and cross-presenting DC. Furthermore, in NOD mice *C. rodentium* accelerated insulitis and upregulated TLR2, TLR4 and CXCL10 expression by islet cells. Our results are in evidence for a direct, dysbiosis-activated route for DC and soluble material from the gut to the PaLN via lymphatics. This route may be the missing link between dysbiosis and its potential effects on T1D.

WS.D1.03.03

Imaging mass cytometry reveals the microanatomical location of B cell subsets in human gut associated lymphoid tissue

T. Tull[1], *K. Todd*[2], *N. Petrov*[2], *R. Ellis*[2], *S. Heck*[2], *M. Bemark*[3], *J. Spencer*[1];
[1]*King's College London, London, United Kingdom,* [2]*Biomedical Research Centre, Guy's and St. Thomas' NHS Trust, London, United Kingdom,* [3]*Mucosal Immunobiology and Vaccine Center, University of Gothenburg, Gothenburg, Sweden.*

Human gut-associated lymphoid tissue (GALT) is important for host immune defence and for maintenance of homeostatic equilibrium throughout the gastrointestinal tract that is rich in antigens from the microbiota. Here we describe the use of imaging mass cytometry to delineate the microanatomical location of B cell subsets within GALT. Frozen sections from 3 human appendixes were stained simultaneously with 13 metal tagged antibodies and ablated using a Fluidigm Hyperion™ imaging mass cytometer. Pixels were extracted from the image and clustered using Cytobank™ software to identify B cell subsets that were re-plotted onto the original image. Marginal Zone B cells in GALT (CD27+IgM+IgD+) were localized on the mucosal aspect of the germinal centre co-aligned with their CD27-IgD+IgM+CD45RB+ precursor population and sparse naïve B cells. Class switched memory B cells formed a separate zone from the marginal zone B cells on the periphery of the lymphoid tissue extending around the T cell zone and up to the follicle associated epithelium (FAE). These data show that marginal zone B cells are present in the gut as in the spleen and that they are microanatomically separate from class switched memory B cells. IgM only B cells coaligned with both marginal zone and memory B cells consistent with data showing that they can be part of both populations. B cells in the FAE and cells expressing FcRL4 at the epithelial boundary were not of a single phenotype. This study demonstrates the power of imaging mass cytometry for accurate tissue mapping.

WS.D1.03.04

De novo fatty acid synthesis during mycobacterial infection is a prerequisite for the function of highly proliferative T cells, but not for dendritic cells or macrophages

L. Berod[1], *P. Stueve*[1], *L. Minarrieta*[1], *C. Hölscher*[2], *T. Sparwasser*[1];
[1]*Twincore, Hannover, Germany,* [2]*Forschungszentrum, Borstel, Germany.*

Mycobacterium tuberculosis (Mtb) is thought to interfere with macrophage lipid metabolism to ensure its persistence. In dendritic cells (DCs), fatty acid synthesis (FAS) has been suggested to permit optimal cytokine production and antigen presentation. We therefore determined the role of fatty acid metabolism in myeloid cells and T cells during BCG or Mtb infection, using genetic models allowing cell-specific deletion of acetyl-CoA carboxylase (ACC)1 and 2 in DCs, macrophages or T cells. Our results demonstrate that FAS is induced in DCs and macrophages upon BCG infection. However, absence of ACC1 or ACC2 did not influence the ability of DCs and macrophages to cope with infection. In contrast, mice with a deletion of ACC1 specifically in T cells fail to generate efficient Th1 responses and succumb early to Mtb infection. In summary, ACC1-dependent FAS is a crucial mechanism in T cells, but not DCs or macrophages, to fight mycobacterial infection.

WS.D1.03.05

Cutting and pasting in the immunoproteome of infected cells

*C. van Els[1], J. van Gaans-van den Brink[1], J. Liepe[2], H. Meiring[3], M. Mishto[4], A. Platteel[5], M. Poelen[1], **A. Sijts**[5];*
[1]Centre for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, Netherlands, [2]MPI for Biophysical Chemistry, Göttingen, Germany, [3]Intravacc, Bilthoven, Netherlands, [4]King's College London, London, United Kingdom, [5]Utrecht University, Utrecht, Netherlands.

Proteasomes play an important role in intracellular protein degradation. Peptide products resulting from proteasome-mediated proteolysis, may enter the MHC class I antigen processing pathway for display on the cell surface. This allows $CD8^+$ T cells to detect and react to intracellular aberrancies, such as infections. Recently, it was discovered that an unexpected large fraction of peptides, presented by cell surface MHC class I molecules on human cells, are spliced (hybrid) peptides generated by **P**roteasome-**C**atalyzed ligation of peptides / '**P**eptide **S**plicing' (**PCPS**). To examine whether spliced peptides are a target of $CD8^+$ T cells responding to infection, i) we have developed an *in silico* reverse immunology-based approach to identify proteasome-generated spliced epitopes within protein sequences. Applying this strategy to a murine *Listeria monocytogenes* infection model, we identified two novel spliced epitopes within one of the secreted bacterial proteins that were recognized by $CD8^+$ T cells of *L. monocytogenes*-infected mice. ii) in parallel, we have applied an immunoproteomics workflow to reveal the spliced compared to linear immunopeptidome presented by MHC class I molecules of Measles Virus infected cells. Mass spectrometry analysis of peptide fractions eluted from affinity-purified MHC class I molecules identified approximately 8000 MHC class I ligands in total, amongst which diverse linear as well as spliced MeV peptide sequences. Taken together, these studies demonstrate that PCPS expands the $CD8^+$ T cell response against intracellular pathogens by exposing spliced epitopes on the cell surface.

WS.D1.03.06

Functional bias of effector memory CD8 T cells underlies the pathogenesis of Hepatitis E Virus in the elderly

***J. Gouilly**[1], F. Abravanel[1,2], E. Bahraoui[1], N. Jabrane-Ferrat[1], J. Izopet[1,2], H. El Costa[1,2];*
[1]Centre de Physiopathologie Toulouse Purpan, Toulouse, France, [2]Laboratoire de Virologie, IFB, CHU Toulouse, Toulouse, France.

The occurrence of severe clinical symptoms upon Hepatitis E virus genotype 3 (HEV-3) infection increases with aging. While the infection is clinically silent in young adults, HEV-3 is often associated with acute icteric hepatitis and liver damage in senior people. Why some elderly patients develop symptomatic hepatitis E infection while others remain asymptomatic is yet to be defined. Herein, we aimed at elucidating the mechanisms underlying age-related pathogenesis of HEV-3 by assessing phenotypic and functional plasticity of CD8 T cells during symptomatic *versus* asymptomatic infection in patients aged 60 and over. Among CD8 T subsets, effector memory cells (EM) were involved in the outcome of HEV-3 infection. Symptomatic patients were characterized by an increased proportion of highly activated EM CD8 T cells expressing HLA-DR and CD38 along with PD-1, TIM-3 and LAG-3. This activation status was associated with loss of polyfunctional cytokine type-1 production and partial commitment of EM compartment to type-2 cells producing IL-4. By contrast, EM CD8 T cells from asymptomatic patients were endowed with moderate activation and efficient secretory function. In conclusion, excessive activation associated with qualitative and quantitative defects in cytokine production within the EM CD8 T cell compartment underlie the clinical complications of HEV-3 infection in the elderly.

WS.D2.01 Molecular properties of innate immune cells

WS.D2.01.01

Non-apoptotic TRAIL function modulates NK cell activity during viral infection

*L. Cardoso Alves, M. D. Berger, N. Kirschke, C. Lauer, N. Corazza, **P. Krebs**;*
University of Bern, Bern, Switzerland.

The role of death receptor signaling for pathogen control and infection-associated pathogenesis is multifaceted and controversial. Here, we show that during viral infection, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) modulates NK cell activity independently of its pro-apoptotic function. In mice infected with lymphocytic choriomeningitis virus (LCMV), *Trail*-deficiency led to improved specific $CD8^+$ T cell responses, resulting in faster pathogen clearance and reduced liver pathology. Depletion experiments indicated that this effect was mediated by NK cells. Mechanistically, TRAIL restricts NK1.1-triggered IFNg production by NK cells. In addition, TRAIL expressed by immune cells positively and dose-dependently modulates IL-15 signaling-induced granzyme B production in NK cells, leading to enhanced NK cell-mediated T cell killing. TRAIL also regulates the signaling downstream of IL-15 receptor in human NK cells.
The function of TRAIL on immune cells was so far confined to the induction of apoptosis on target cells. Our study reveals a hitherto unappreciated immunoregulatory role of TRAIL signaling on NK cells for the granzyme B-dependent elimination of antiviral T cells.

WS.D2.01.02

Recognition of host Clr-b by the inhibitory NKR-P1B receptor provides a basis for missing-self recognition

***G. R. Balaji**[1,2], O. A. Aguilar[3,4], Z. Fu[1,2], M. Tanaka[3], J. R. Carlyle[3], J. Rossjohn[1,2,5], R. Berry[1,2];*
[1]Infection and Immunity Program and Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Melbourne, Australia, [2]ARC Centre of Excellence in Advanced Molecular Imaging, Monash University, Melbourne, Australia, [3]Department of Immunology, University of Toronto, and Sunnybrook Research Institute, Toronto, Canada, [4]Present address: Department of Microbiology and Immunology, University of California, San Franciso, United States, [5]Institute of Infection and Immunity, School of Medicine, Cardiff University, Cardiff, United Kingdom.

The interaction between natural killer (NK) cell inhibitory receptors and their cognate ligands constitutes a key mechanism by which healthy tissues are protected from NK cell-mediated lysis. However, self-ligand recognition remains poorly understood within the prototypical NKR-P1 receptor family. Here, we report the structure of the inhibitory NKR-P1B receptor bound to its cognate host ligand, C-type lectin-related-b (Clr-b). NKR-P1B and Clr-b interacted via a head-to-head docking mode through an interface that included a large array of polar interactions. NKR-P1B:Clr-b recognition was extremely sensitive to mutations at the heterodimeric interface, the majority of which severely impacted both Clr-b binding and NKR-P1B receptor function, in line with the low affinity of the interaction. Within the structure, two NKR-P1B:Clr-b complexes were cross-linked by a novel NKR-P1B homodimer, the disruption of which abrogated Clr-b recognition. Altogether, these data provide an insight intoa fundamental missing-self recognition system and suggests an avidity-based mechanism underpins NKR-P1B receptor function.

WS.D2.01.03

CD16A activation of NK cells primes memory-like cytotoxicity against cancer cells

***J. Pahl**[1,2], J. Koch[3], J. Götz[2], A. Arnold[2], U. Reusch[3], T. Gantke[3], E. Rajkovic[3], M. Treder[3], A. Cerwenka[1,2];*
[1]Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany, [2]German Cancer Research Center, Heidelberg, Germany, [3]Affimed GmbH, Heidelberg, Germany.

CD16A/FcγRIIIa is a potent cytotoxicity receptor on human NK cells, which can be exploited by therapeutic antibodies to target and eliminate cancer cells by NK cells. So far, the effects of CD16A-mediated activation on NK cell effector functions beyond classical antibody-dependent cytotoxicity have remained poorly elucidated. Here, we investigated how tumor-reactive therapeutic antibodies such as bispecific AFM13 (CD30/CD16A) or anti-CD20 rituximab modulate the functionality of NK cells and their subsequent responsiveness to cytokines and tumor cells. Our results reveal that CD16A engagement by therapeutic antibodies greatly enhanced IL-2 and IL-15-driven NK cell proliferation and expansion. This effect involved up-regulation of CD25 (IL-2Rα) and CD132 (γ_c) on NK cells, resulting in increased sensitivity to low-dose IL-2 or to IL-15. CD16A engagement initially induced NK cell cytotoxicity, while one day later NK cell reactivity was reduced, which could be recovered by subsequent re-culture with IL-2 or IL-15. Remarkably, after re-culture, CD16A-experienced NK cells exerted more vigorous IFN-γ production upon re-stimulation with tumor cells or cytokines. Importantly, CD16A-experienced NK cells exerted increased cytotoxicity towards different tumor targets, mainly through the activating NK cell receptor NKG2D. Our findings uncover a novel role for CD16A in priming enhanced NK cell responses to re-stimulation by cytokines and tumor cells, indicative of a memory-like functionality. Altogether, a treatment combination of CD16A-engaging antibodies with IL-2 or IL-15 may boost NK cell anti-tumor activity in patients by expanding tumor-reactive NK cells and enhancing NK cell reactivity even upon repeated tumor encounters.

WS.D2.01.04

Dynamic changes in intrathymic ILC populations during neonatal development

***R. Jones**, G. Anderson, D. R. Withers;*
University of Birmingham, Birmingham, United Kingdom.

Introduction: Members of the innate lymphoid cell (ILC) family have been implicated in the development of thymic microenvironments and the recovery of this architecture after damage. Despite this, a robust characterisation of the ILC populations present in thymus is lacking and identification of ILCs amongst developing thymocytes is technically challenging given the close phenotypic similarities of ILC and T cells. Objective: To better understand the ILC compartment of the thymus. Methods: We have utilised multiple in vivo models to robustly characterise this population, including the fate mapping of Id2 expression and the use of Id2 reporter mice. Results: Our data demonstrate that ILCs are most prominent immediately after birth, but were rapidly diluted as the T cell development programme is increased. As observed in the embryonic thymus, $CCR6^+NKp46^-$ lymphoid tissue inducer cells were the main ILC3 population present, but numbers of these cells swiftly declined in the neonate and these cells were barely detectable in adult thymus.

This loss of ILC3 helps establish GATA-3^{+} ILC2 as the dominant ILC population in the thymus, with numbers of these cells gradually increasing during neonatal development. Interestingly, ILC2 were located in the medulla and in neonatal mice lacking ILC3 were able to provide RANK-L; suggesting potential competition between ILC populations for niches within the thymus. Conclusions: Collectively, these data reveal a developmental switch in the ILC populations of the thymus, with ILC3 contributing to establishing the thymic microenvironment in the embryo, but after birth ILC2 are the dominant ILC population.

WS.D2.01.05
Innate lymphoid cell reconstitution post lethal irradiation and bone marrow transplant

R. ALBUGAMI, *R. Alhamawi, C. Dempsey, C. Willis, D. Withers, N. Jones;*
Institute of Biomedical Research, BIRMINGHAM, United Kingdom.

Innate lymphoid cells (ILCs) are a family of immune cells which can be subdivided into group 1, group 2 and group 3 ILCs according to the expression of signature transcription factors and cytokines. They share the same morphology as B and T lymphocytes; however, they lack rearranged antigen receptors. Data from human and mouse studies suggest that ILCs can influence the development and severity of graft-versus-host disease (GVHD), a common complication of allogeneic bone-marrow transplantation (BMT). Therefore, determining the kinetics of reconstitution and phenotype of ILCs post-BMT is a crucial first step to manipulating these cells for therapeutic benefit. Following lethal irradiation of CD45.2+BALB/c recipients and adoptive transfer of CD45.1+C57BL/6 T cell depleted bone marrow, we found that recipient ILCs initially survived in non-lymphoid tissues post-irradiation but were slowly lost with time. Interestingly, despite full reconstitution of other immune cells by 60 days post-BMT, donor-derived ILCs remained severely depleted 11.8%, 17.7%, 11.6% of WT controls in small intestine, spleen and mLN, respectively (n=4).
In stark contrast, when performing BMT from BALB/c to CD45.1+ C57BL/6, donor-derived ILCs were found to fully reconstitute in all tissues (small intestine=202.9%, spleen=151.6% and mLN=235.9% compared to WT controls (n=4). These data suggest that ILCs can reconstitute all non-lymphoid and lymphoid compartments following BMT however this may be dependent on a number of factors. We will discuss the potential role of the environment (microbiota - immune system interactions), strains and sex differences of BMT recipients all of which are currently being investigated by the laboratory.

WS.D2.01.06
Killer cell Immunoglobulin-like receptor 3DL1 polymorphism defines distinct hierarchies of antigen recognition

J. P. Vivian[1], *P. Saunders*[2], *P. Pymm*[1], *J. Rossjohn*[1], *A. Brooks*[2];
[1]*Monash University, Clayton, Australia,* [2]*Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, The University of Melbourne., Melbourne, Australia.*

The interaction between killer cell Immunoglobulin-like Receptor 3DL1 (KIR3DL1) and HLA-class I molecules has been linked to NK cell control of viral infections and malignancy. However due to the vast polymorphism of the genes encoding both its HLA-I ligands and the receptor itself, a mechanistic understanding of how these receptor/ligand interactions impact disease outcomes remains unclear. KIR3DL1 tetramers representative of the two main inhibitory KIR3DL1 lineages (-*005 and -*015) together with an interlineage recombinant (*001) were screened for reactivity against a comprehensive panel of HLA-I molecules. This revealed distinct hierarchies of preferred HLA-I ligands for each KIR3DL1 allotype, with KIR3DL1*005 recognising a wider array of HLA-I ligands than either the KIR3DL1*015 or -*001. These differences in binding were also reflected in functional assays utilising NK cell clones expressing specific KIR3DL1 allotypes. Intriguingly, while the polymorphic differences between KIR3DL1*001, *005 and *015 were remote from the KIR3DL1-HLA-I interface, the structures of the three KIR3DL1-HLA-I complexes showed that the broader specificity of KIR3DL1*005 correlated with an altered juxtapositioning of the D1-D2 domains and increased mobility within the ligand binding site, conferring a greater tolerance for disparate ligands. Collectively, we provide a molecular basis underpinning the impact of KIR3DL1 polymorphism on HLA-I recognition that has important implications for haematopoietic transplantation. Reference: Saunders P. and Vivian J.P., *et al* Journal of Experimental Medicine. (2016) 213: 791-807.

WS.D2.02 Molecular and cellular features of ILCs

WS.D2.02.01
Hobit specifically identifies ILC1 throughout peripheral tissues

R. L. R. Taggenbrock[1], *N. A. Kragten*[1], *F. M. Behr*[1,2], *L. Parga Vidal*[1], *T. H. Wesselink*[1], *R. Stark*[1,2], *G. Gasteiger*[3], *K. P. van Gisbergen*[1,2];
[1]*Sanquin research and Landsteiner laboratory, Amsterdam, Netherlands,* [2]*Department of Experimental Immunology AMC, Amsterdam, Netherlands,* [3]*Institute of Systems Immunology, University of Würzburg, Würzburg, Germany.*

Innate lymphoid cells (ILCs) form populations of lymphocytes involved in the regulation of inflammation, tissue repair, and immune homeostasis. These ILCs are present in mucosal barrier tissues such as the lamina propria and epithelium of the small intestine, the lungs and the salivary glands. Different subsets of ILCs have been recognized including ILC1, ILC2 and ILC3 that contribute to immune responses through the production of different sets of cytokines. The lack of ILC-specific tools has hampered the study of ILC development. Previously, we have shown that the transcription factor Homologue of Blimp-1 in T-cells (Hobit) regulates the maintenance of ILC1s, but not of conventional NK cells in the murine liver. We now have developed Hobit Reporter mice, containing a “knock in” of the fluorescent protein tdTomato and CRE recombinase in the Hobit locus that allows us to specifically address the differentiation of ILC1. We analyzed tdTomato expression in ILC subsets of the Hobit reporter mice and found high levels of Hobit expression in ILC1s in several peripheral organs including liver, small intestine and salivary gland. In contrast, conventional NK cells, ILC2 and ILC3 in these organs did not express Hobit. Using Hobit Cre x Rosa26 (floxed STOP) YFP lineage tracer mice, YFP expression was found in tdTomato+ ILC1, but not in tdTomato- NK cells, ILC2 and ILC3, suggesting that ILC1 do not contribute to the differentiation of other ILC lineages. We conclude that Hobit identifies ILC1 as terminally differentiated cells that are maintained as an independent population in the periphery.

WS.D2.02.02
Human IL-17-producing type 2 innate lymphoid cells are important regulators of neutrophilia in cystic fibrosis

K. Golebski, X. R. Ros, M. Nagasawa, B. A. Heesters, C. M. van Drunen, W. J. Fokkens, H. Spits, ***S. M. Bal****;*
Academic Medical Center, Amsterdam, Netherlands.

Innate lymphoid cells (ILCs) are crucial in the immune surveillance at mucosal sites. They coordinate early eradication of pathogens and contribute to tissue healing and remodelling, features that are dysfunctional in patients with cystic fibrosis (CF). ILCs have been implicated in CF, but the mechanisms by which they contribute to CF-immunopathology remain ill-defined.
Here we compared the ILC distribution and cytokine production in nasal polyps (NP) from CF and chronic rhinosinusitis patients and performed in vitro cultures with and without NP epithelial cells to study the mechanism behind ILC activation in CF inflammation. Finally, transcriptome analysis was performed on ILC2s cultured under conditions that induced different cytokine production profiles.
NP from CF patients were enriched with IL-17-producing ILCs. Unexpectedly, this was a result of group 2 ILCs (ILC2s) transdifferentiating from IL-5-producing into IL-17-secreting cells in response to epithelial derived IL-1β, IL-23 and TGF-β. These cytokines were elevated in NP from CF patients and induced upon infection with *Staphylococcus aureus* or *Pseudomonas aeruginosa*. IL-17-producing ILC2s induced IL-8 secretion by epithelial cells and their presence correlated with NP neutrophilia. IL-17-producing ILC2s expressed the transcription factor RORγt and could be identified by surface expression of CCR4 and CCR6. Finally, we show that this conversion of ILC2s could be abrogated by IL-4 or vitamin D3. Our data support a model in which ILC2s undergo transdifferentiation in response to local cytokines and show that limiting ILC plasticity is a potential target to ameliorate CF inflammation.

WS.D2.02.03
ILC migration between barriers sites and draining lymphoid tissue

E. Dutton[1], *C. Willis*[1], *A. Camelo*[2], *M. Sleeman*[3], *G. Carlesso*[4], *R. Herbst*[5], *A. Humbles*[2], *D. Withers*[1];
[1]*University of Birmingham, Birmingham, United Kingdom,* [2]*MedImmune, Cambridge, United Kingdom,* [3]*Regeneron, Tarrytown, United States,* [4]*MedImmune, Gaitherburg, United States,* [5]*MedImmune, Gaithersburg, United States.*

Parabiosis studies argue that most ILCs permanently reside within mucosal tissues. The extent to which ILCs are resident within lymphoid tissues is unclear and we hypothesised that ILC migration between skin and peripheral lymph nodes would be enhanced under conditions of local inflammation. Photoconversion of the brachial LN (bLN) of Kaede transgenic mice demonstrated that the ILC compartment of bLNs was not fixed and whilst ILC are far less migratory than T cells, lymph nodes contain both resident and migratory ILC populations. Analysis of CCR7$^{-/-}$Kaede mice indicated that ILC entry into tissue was CCR7-dependent, whilst analysis of contralateral LNs demonstrated minimal recirculation of these cells through lymph nodes. To determine whether ILCs migrate into peripheral LNs from the skin, photoconversion of the ear was used to assess the migration of ILCs between the ear and draining auricular LN (auLN). Under steady state there was little evidence of ILC migration to the auLN or into the labelled area of skin. However, under conditions of MC903-induced atopic dermatitis (AD), ILC migration to the draining lymph node was evident, although local proliferation alongside traffic from the circulation likely accounted for much of the increase in ILC number within the reactive lymph node. Interestingly, AD induced ILC migration into the inflamed tissue and using CCR6$^{-/-}$ Kaede mice we showed that this migration was CCR6-dependent. Thus the ILC populations in peripheral lymph nodes are not fixed and changes in these populations, in part through migration, may contribute to shaping adaptive immunity at barrier sites.

WS.D2.02.04

The chemokine receptor CCR8 regulates effector functions of group 2 innate lymphoid cells

***L. Knipfer**, A. Schulz-Kuhnt, M. Kindermann, T. Primbs, D. Vöhringer, M. Neurath, I. Atreya, S. Wirtz;*
University Clinic Erlangen, Erlangen, Germany.

In recent years, group 2 innate lymphoid cells (ILC2s) were revealed to possess an indispensable role during helminthic infections as well as acute and chronic airway inflammatory diseases. While their immunoregulatory capacity is known to be largely mediated by the production of type 2 cytokines, the factors and mechanisms involved in migration and tissue tropism of ILC2s are poorly understood. Given that our initial data showed that the type 2 immune response-associated chemokine receptor CCR8 is within the ILC subsets specifically expressed in both human and mouse ILC2s at the mRNA and protein level, we analyzed the role of CCR8 in the well characterized *Nippostrongylus brasiliensis* infection model. We found that CCR8$^{-/-}$ mice displayed substantially diminished lung ILC2 counts at day 9 post infection, which was accompanied by decreased amounts of key type 2 effector cytokines, especially IL-9. Moreover, these mice showed significantly less eosinophilia and were overall impaired in their capacity to expel adult worms from their intestines. However, migration assays and lightsheet microscopy analysis with wildtype and CCR8$^{-/-}$ ILC2s clearly indicated that ILC2s did not migrate towards CCR8 ligands in vitro and in vivo. By contrast, in vitro stimulation experiments with the CCR8 ligand CCL1 and specific CCR8 inhibitors identified CCR8-signalling as an important factor regulating the growth and effector cytokine production of mouse and human ILC2s. These data add to our understanding of ILC2 biology and may also support the further development of clinical approaches, as CCR8 is a promising target for monoclonal antibody therapy of asthma.

WS.D2.02.05

Molecular definition of group 1 innate lymphoid cells in the mouse uterus

***I. Filipovic**[1,2,3], L. Chiossone[4], P. Vacca[5], R. S. Hamilton[1], J. Doisne[6], A. Sharkey[7], C. Mingari[8], L. Moretta[9], F. Colucci[1,2];*
[1]Centre for Trophoblast Research, University of Cambridge, Cambridge, United Kingdom, [2]Department of Obstetrics and Gynaecology, University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom, [3]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom, [4]G. Gaslini Institute, Genoa, Italy, [5]Department of Experimental Medicine (DIMES), University of Genoa,, Genoa, Italy, [6]Department of Immunology, Pasteur Institute, Paris, France, [7]Department of Pathology, University of Cambridge, Cambridge, United Kingdom, [8]Department of Experimental Medicine (DIMES), University of Genoa, Genoa, Italy, [9]Department of Immunology, IRCCS Bambino Gesù Children's Hospital, Rome, Italy.

Determining the function of uterine lymphocytes is challenging because of the rapidly changing nature of the organ in response to sex hormones and, during pregnancy, to the invading fetal trophoblast cells. Here we provide the first genome-wide transcriptome atlas of mouse uterine group 1 innate lymphoid cells (g1 ILCs) at mid-gestation. The subset composition of g1 ILCs fluctuates throughout reproductive life, with Eomes^{-ve}CD49a^{+} cells dominating before puberty and specifically expanding in second pregnancies, when the expression of CXCR6, a marker associated with memory NK cells, is upregulated. Tissue-resident Eomes^{+}CD49a^{+} NK cells (trNK), which resemble human uterine NK cells, are most abundant during early pregnancy, and showcase gene signatures of responsiveness to TGF-β and IL-1, and connections with trophoblast, epithelial, endothelial and smooth muscle cells, leucocytes, as well as extracellular matrix. Tissue-resident NK cells express genes involved in aerobic glycolysis, lipid metabolism, iron transport, protein ubiquitination, and recognition of microbial molecular patterns. Conventional NK cells expand late in gestation and may engage in crosstalk with trNK cells involving IL-18 and IFN-γ. These results identify trNK cells as the cellular hub of uterine g1 ILCs at mid-gestation and mark Eomes-veCD49a+CXCR6+ g1 ILCs as potential memory cells of pregnancy. This work is supported by grants from the Wellcome Trust and Centre for Trophoblast Research.

WS.D2.02.06

NCR+ ILC3 maintain larger STAT4 reservoir via T-BET to regulate type 1 features upon IL-23 stimulation in mice.

*Y. Mikami[1], G. Scarno[2], B. Zitti[3], H. Shih[1], Y. Kanno[1], A. Santoni[2], J. J. O'Shea[1], **G. Sciume**[2];*
[1]National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, MD, United States, [2]Sapienza, University of Rome, Rome, Italy, [3]HERM, Department of Medicine, Karolinska Institutet, Stockholm, Sweden.

Type 3 innate lymphoid cells (ILC3) represent a heterogeneous group of cells producing interleukin(IL)-22 and/or IL-17, and involved in regulation of gut barrier homeostasis and inflammation. Cytokines activating the Janus kinases (Jaks) and members of the signal transducer and activator of transcription (STAT) pathway are key players in lymphoid development, differentiation and activation. In this context, we have previously dissected the role of STAT5 in regulation of ILC homeostasis in mice and revealed that ILC3 expressing natural cytotoxicity receptors (NCR+ ILC3) are particularly sensitive to deprivation of STAT5 signals. Here, by using mouse models in combination with genomic and transcriptomic approaches, we defined at the molecular level, initial events of NCR+ ILC3 activation and the contribution of STAT4 in the acquisition of type 1 features, downstream of IL-23 stimulation. In particular, we observed high basal expression of STAT4 in NCR+ ILC3, dependent on T-BET, which contributes to enhance production of IFN-γ. Altogether, our findings shed light on a feed-forward mechanism involving STAT4 and T-BET that modulates the outcome of IL-23 signaling in ILC3.

WS.D3.01 Novel vaccine approches to intracellular pathogens

WS.D3.01.01

Targeted delivery of BCG vaccine to the DEC-205 receptor improves protective efficacy against *Mycobacterium tuberculosis*

***C. Counoupas**[1], R. Pinto[1], L. Baker[2], G. Ngalingam[1], A. Nisa[1], W. Britton[1], J. Triccas[2];*
[1]Centenary Institute, Sydney, Australia, [2]University of Sydney, Sydney, Australia.

Tuberculosis (TB) remains a major cause of mortality and morbidity worldwide, with 1.7 million deaths annually and a third of the world infected with the latent form of the disease. The currently available vaccine, *M. bovis* BCG, is only partially effective against TB, and new strategies are required to control the global epidemic. We hypothesised that BCG may interact sub-optimally with dendritic cells (DCs), the cell type pivotal in shaping the adaptive immune response to infectious agents. Thus strengthening this interaction could improve BCG protective efficacy. In order to do this, we engineered BCG to express the single-chain variable fragment (ScFv) recognising the C-type lectin receptor DEC205 (BCG:DEC), which is expressed predominantly on migratory DCs. An increased functional ability of BCG:DEC to interact with DEC205-expressing cell lines and bone morrow derived DCs was observed; interestingly, the increased interaction resulted in an augmented secretion of inflammatory cytokines and chemokines by BMDCs. Given the high levels of expression of DEC205 in the skin of mice, we tested the protective efficacy of BCG:DEC after subcutaneous vaccination. We found that BCG:DEC conferred greater protection than BCG 4 weeks after aerosol challenge with virulent *M. tuberculosis,* and its efficacy lasted up to 20 weeks post infection, even when BCG protection had waned. Our results indicate that targeting DCs by modifying BCG is an effective strategy to improve protection against TB.

WS.D3.01.02

HLA-E restricted human CD8^{+} T-cells: a novel human T-cell subset with unique properties which impact vaccination strategies against tuberculosis

***S. A. Joosten**, K. E. van Meijgaarden, T. Prezzemolo, A. M. Drittij, K. L. Franken, C. K. Dingenouts, P. Ruibal, T. H. Ottenhoff;*
Leiden University Medical Center, Leiden, Netherlands.

Mycobacterium tuberculosis (Mtb) infection remains a major global threat, and better vaccines are urgently required. BCG induces only limited protection and novel vaccination strategies are thought to be crucial to eliminate tuberculosis (TB). HLA-E presented antigens are interesting targets for vaccination given HLA-Es' essentially monomorphic nature. HLA-E is a member of the HLA class Ib family and can present both self-peptides as well as peptides derived from intracellular pathogens. The precise phenotype and functional capacity of these cells remains poorly characterized. We have developed a new protocol combining HLA-E tetramer with intracellular staining for cytokines, transcription factors and cytotoxic molecules to characterize these cells in depth. Moreover, we have assessed the capacity of these HLA-E restricted T-cells to control intracellular Mtb outgrowth and the mechanism employed. The *ex vivo* frequency of Mtb peptide/ HLA-E-TM^{+} CD8^{+} T-cells was significantly increased in the circulation of TB patients, which are even further increased in those with concomitant HIV. HLA-E restricted CD8^{+} T-cells from TB patients produced more IL-13 than cells from controls or subjects with latent tuberculosis infection (LTBI). Compared to total CD8^{+} T-cells, HLA-E restricted cells produced more IFNγ, IL-4, IL-10, and granulysin but less granzyme-A. Moreover, compared to "classical" Mtb-specific HLA-A2 restricted CD8^{+} T-cells, HLA-E restricted CD8^{+} T-cells produced less TNFα and perforin, but more IL-4. Moreover, HLA-E restricted T-cells could inhibit intracellular growth of Mtb. Thus HLA-E restricted-Mtb specific cells represent a unique new human T-cell subset and are a novel target for vaccination against infectious diseases.

WS.D3.01.03

During experimental tuberculosis, interleukin-27R regulates protection and limits the expansion of multifunctional T cells by inhibiting interleukin-17A

K. Ritter[1], *J. Behrends*[2], *A. Hölscher*[1], *J. Volz*[1], *I. Rosenkrands*[3], *H. Erdmann*[1], *C. Hölscher*[1];
[1]*Infection Immunology, Research Center Borstel, Borstel, Germany,* [2]*Fluorescence Cytometry Core Unit, Research Center Borstel, Borstel, Germany,* [3]*Department of Infectious Disease Immunology, Statens Serum Institut, Copenhagen, Denmark.*

After infection with *Mycobacterium tuberculosis* (Mtb), mice lacking the IL-27R exhibit lower bacterial burdens but develop immunopathological sequelae in comparison to wildtype mice. This phenotype correlates with an enhanced recruitment of antigen-specific $CCR6^+$ $CD4^+$ T cells and an increased frequency of IL-17A-producing $CD4^+$ T cells. By comparing the outcome of Mtb-infection in C57BL/6, IL-27R-deficient and IL-27R/IL-17A-double deficient mice, we observed that in the absence of IL-27R both the increased protection and elevated immunopathology is supported by IL-17A. Whereas IL-17A does neither impact the development of Tr1 cells nor the expression of PD1 and KLRG1 on T cells in IL-27R-deficient mice during infection, it significantly regulates the presence of multifunctional T-cells, co-expressing IFN-γ, IL-2 and TNF, and highly stratified granulomas in the lungs. In addition, IL-17A supports *Cxcl9*, *Cxcl10* and *Cxcl13* expression in lungs of infected IL-27R-deficient mice. Taken together, IL-17A contributes to protection in Mtb-infected IL-27R-deficient mice probably through a chemokine-mediated recruitment and strategic positioning of multifunctional T cells in granulomas. Although IL-27 also prevents IL-17A-mediated immunopathology, a timely inhibition of IL-27R-mediated signaling during vaccination or host-directed therapy of tuberculosis might increase a coordinated protective effect of IL-17A without detrimental consequences.

WS.D3.01.04

Human *Plasmodium falciparum* infection induces a predictable and persistent distortion in transcriptomic landscapes across T cell subsets

T. S. Watkins[1,2,3], *F. Amante*[2], *S. Turner*[2], *S. Darko*[4], *A. Ransier*[4], *D. L. Doolan*[1,2], *D. C. Douek*[4], *J. S. McCarthy*[2,3], *J. J. Miles*[1,2,3];
[1]*AITHM, James Cook University, Cairns, Australia,* [2]*QIMR Berghofer, Brisbane, Australia,* [3]*University of Queensland, Brisbane, Australia,* [4]*VRC, NIH, Bethesda, United States.*

Malaria infection still remains one of the world's most formidable issues across both health and economical systems. Despite the best intervention strategies over 200 million people are infected annually, and mortality rates exceed 400,000 deaths per year. To develop effective therapeutics and vaccines a better understanding of fundamental human immunology is required. This is particularly true of the contribution of T cells to malaria defence. Research to date comes from field samples which can sometimes be difficult to interpret due to confounding factors including multiple exposures. As such, the underlying immune correlates of an efficient response remain unknown. To investigate the role of T cells in disease we used a human model of blood stage *Plasmodium falciparum* infection involving the Medicines for Malaria Venture (MMV).
We performed high-dimensional phenotyping and T cell receptor (TCR) sequencing of T cell subsets before infection, during infection and during convalescence. *Plasmodium falciparum* infection resulted in marked phenotypic 'scaring' across all T cell subsets and persisted during convalescence. We identified gene modules that correlated with parasitaemia, revealing a possible continua between individuals at risk of disease and 'elite controllers'. Remodelling of the TCR repertoire could also be correlated with transcriptomic changes, and gene expression and TCR repertoire metrics could serve as novel proxies of disease burden and assist in identifying pathways for immunotherapies or vaccine activation. Indeed, characterizing the T cell biology of elite controllers opens the possibility of inducing this phenotype for the purposes of rational vaccine design and therapeutic intervention.

WS.D3.01.05

BCG-induced trained innate immunity during controlled human malaria infection

J. Walk[1], *C. de Bree*[1], *W. Graumans*[1], *R. Siebelink-Stoter*[1], *G. van Gemert*[1], *M. van de Vegte-Bolmer*[1], *K. Teelen*[1], *C. C. Hermsen*[1], *R. J. Arts*[1], *M. C. Behet*[1], *S. J. Moorlag*[1], *A. Yang*[1], *R. van Crevel*[1], *P. Aaby*[2], *Q. de Mast*[1], *A. J. van der Ven*[1], *C. Stabell Benn*[2], *M. Netea*[1], *R. W. Sauerwein*[1];
[1]*Radboud university medical center, Nijmegen, Netherlands,* [2]*Statens Serum Institut, Copenhagen, Denmark.*

Recent evidence suggests that certain vaccines, including Bacillus-Calmette Guérin (BCG), can induce changes in the innate immune system with non-specific memory characteristics, termed 'trained immunity'. We performed a randomized, controlled clinical trial in twenty healthy male and female volunteers to evaluate the induction of immunity and protective efficacy of BCG vaccination against a controlled human malaria infection (CHMI) five weeks after vaccination. BCG vaccinated volunteers had earlier and more severe clinical symptoms, and showed a heterologous, memory-like monocyte and (innate) lymphocyte re-activation that correlated with reduced parasitemia. These findings demonstrate for the first time that BCG vaccination induces trained immunity with functional activity against an unrelated, clinically relevant, human pathogen *in vivo*. It forms a strong impetus to further explore its potential in the clinical development of a rational malaria vaccine strategy.

WS.D3.01.06

Evaluation of multi-epitope peptides derived from different *Leishmania* proteins as potential vaccine candidates against human Leishmaniasis

S. Hamrouni[1], *K. Aoun*[1], *A. Kidar*[2], *R. Chamakh Ayari*[1], *E. Petitdidier*[3], *J. Lemesre*[3], *R. Bras Gonçalves*[3], *A. Meddeb Garnaoui*[1];
[1]*Institut Pasteur, Tunis, Belvédère, Tunisia,* [2]*Hôpital régional, Gafsa, Tunisia,* [3]*Institut de Recherche pour le Développement, Montpellier, France.*

IFN-γ-producing-Th1 cells are required for *Leishmania* parasite control. Healing is correlated with resistance to reinfection, suggesting that vaccination is feasible. To date no vaccine is available.
To identify peptide vaccine candidates against human leishmaniasis, we evaluated the immunogenicity of multi-epitope peptides in individuals with healed cutaneous leishmaniasis due to *Leishmania major*.
PSA (Promastigote Surface Antigen), LmlRAB (*L. major* large RAB GTPase) and H2B (Histone), reported to be immunogenic in humans, mice and dogs, were screened *in silico* for T cell epitopes able to bind to frequent HLA class-I and -II alleles. Selected peptides were synthesized, pooled and used to screen peripheral blood mononuclear cells from subjects cured of cutaneous Leishmaniasis. Healthy subjects were used as control group. IFN-γ and IL-10 production was evaluated by ELISA. Phenotypes of cytokines producing T cells were analyzed by flow cytometry.
We showed that some peptide pools were able to elicit significant IFN-γ levels in cured but not in healthy individuals. No IL-10 production was detected.
A significant increase in the percentage of IFN-γ-producing CD4+ T cells and polyfunctional CD4+T cells producing IFN-γ, TNF-α and IL-2 was detected in response to peptide pools.
A significant increase of the percentage of CD4+CD45RO+CCR7+central memory T cells was also observed.
In conclusion, we demonstrated that multi-epitope peptides derived from different *Leishmania* proteins were able to induce IFN-γ and polyfunctional CD4+T cells, both associated with protection against *Leishmania* infection, suggesting that they may be exploited as vaccine candidates.
NVVI Travel Grants for Early Career Scientist from Developing Countries

WS.D3.02 Novel vaccine approaches for viruses

WS.D3.02.01

Deciphering the complexity of vaccine-induced immunity with omics technologies: innate immune responses differ between priming and boosting immunization

J. L. Palgen[1,2], *N. Tchitchek*[1,2], *J. Elhmouzi-Younes*[1,2], *S. Delandre*[1,2], *I. Namet*[1,2], *N. Huot*[2,3], *P. Rosenbaum*[1,2], *N. Dereuddre-Bosquet*[1,2], *F. Martinon*[1,2], *A. Cosma*[1,2], *M. Müller-Trutwin*[2,3], *Y. Lévy*[2,4], *R. Le Grand*[1,2], *A. S. Beignon*[1,2];
[1]*CEA – Université Paris Sud 11 – INSERM U1184, Immunology of Viral Infections and Autoimmune Diseases, IDMIT department, IBFJ, Fontenay-aux-Roses, France,* [2]*Vaccine Research Institute, Créteil, France,* [3]*Institut Pasteur, Unit on HIV, Inflammation and Persistence, Paris, France,* [4]*Institut Mondor de Recherche Biomédicale – INSERM U955, équipe 16 physiopathologie et immunothérapies dans l'infection VIH, Créteil, France.*

A better understanding of vaccine-induced innate responses and their impact on immune memory is critical to more efficiently design long-term protective vaccines, but the immune system complexity admittedly makes it challenging. To take up this challenge, omics technologies are promising since they allow multiparametric and multiscale measurements.
Here, we used mass cytometry and DNA micro-arrays to longitudinally study blood innate cells (granulocytes, monocytes, DC and NK cells) during prime-boost vaccination of cynomolgus macaques with the Modified Vaccinia virus Ankara (MVA) attenuated vaccine. We developed new analyses pipelines to explore those datasets and assess the magnitude, quality and dynamics of immune responses after each immunization.
Innate myeloid and lymphoid cells numbers were similarly early, transiently and strongly impacted after each immunization. However, at a deeper resolution, several subphenotypes were differently enriched/attrited between each immunization. The key features of prime-boost differences, identified using multivariate analyses, include the differential expression of CD11b, CD66, CD45 and CD32 on neutrophils; CCR5, CXCR4, CD45 and CD16 on cDCs; CD32, CD11a, CD14 and CD45 on monocytes; and CD16, CCR5, CD69 and CD107a on NK cells. More strikingly, before the boost, the subphenotype composition of innate cells differed from baseline with the upregulation of those activation/maturation markers, strongly suggesting that the innate immune system is imprinted after the prime.
This work indicates that, similarly to adaptive responses, innate responses differ after each vaccine encounter. This revisits vaccine-induced innate responses and open new perspectives with the involvement of innate training/memory.

WS.D3.02.02

Identification of vaccine responding T-cells using TCR repertoire sequencing data

M. V. Pogorelyy[1,2], *A. A. Minervina*[1], *M. Puelma Touzel*[3], *E. A. Komech*[1], *E. I. Kovalenko*[1], *G. G. Karganova*[4], *E. S. Egorov*[1], *A. Y. Komkov*[1], *D. M. Chudakov*[1], *I. Z. Mamedov*[1], *T. Mora*[5], *A. M. Walczak*[6], *Y. B. Lebedev*[1];
[1]*Shemyakin–Ovchinnikov Institute of bioorganic chemistry RAS, Moscow, Russian Federation,* [2]*Pirogov Russian National Research Medical University, Moscow, Russian Federation,* [3]*Laboratoire de physique théorique de l'École Normale Supérieure, PSL University, CNRS, Sorbonne Universités, UPMC, Paris, France,* [4]*Chumakov Institute of Poliomyelitis and Viral Encephalitides, Moscow, Russian Federation,* [5]*Laboratoire de physique statistique, CNRS, Sorbonne Université, Université Paris-Diderot, and École normale supérieure (PSL), Paris, France,* [6]*Laboratoire de physique théorique, CNRS, Sorbonne Université, and École normale supérieure (PSL), Paris, France.*

Introduction: High-throughput sequencing allows for deep profiling of TCR repertoires, however our ability to extract clinically relevant information from this data is limited. We develop approaches to identify clonotypes participating in immune response from both longitudinal repertoire data and single snapshot of repertoire.
Methods: We collected PBMCs, CD4+, CD8+ T-cells at 5 timepoints before and after yellow fever immunization of 3 pairs of twins. We sequenced TCR repertoires from all samples on illumina HiSeq.
Results: Using a novel statistical approach, we identified 500-1500 clones expanded at the peak of the response (day 15) in each individual, occupying up to 5% and 8% of CD4+ and CD8+ repertoires. We validated these clonotypes by an IFN-gamma secretion assay, MHC-multimer staining and sorting of activated T-cell subpopulations. We found little sequence overlap even between YF-reactive repertoires of identical twins, but lots of YF-reactive TCR sequences in different donors were homologous. We also developed an algorithm to identify clusters of unlikely similar TCR clonotypes in single repertoire samples. Such clusters were almost absent before immunization and very abundant at the peak of the response, where they mostly consisted of YF-specific sequences (40%-75%). We also found such clusters (immune response signatures) in published immunotherapy and autoimmune repertoire sequencing data.
Conclusions: T-cell response to YF-vaccine is strong and almost unique even in identical twins. Highly homologous sequences in antigen-specific repertoires allow us to build an algorithm to identify clusters of similar clonotypes participating in an active immune response in single repertoire samples.
Supported by RSF-15-15-00178

WS.D3.02.03

Mucosal vaccine mediates cross-protection by immune training

P. Brandi[1], *L. Conejero*[2,1], *F. Cueto*[1], *S. Martinez-Cano*[1], *P. Saz-Leal*[1], *M. Enamorado*[1], *J. Amores-Iniesta*[1], *J. Subiza*[2], *D. Sancho*[1];
[1]*Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain,* [2]*Inmunotek S.L., Madrid, Spain, Madrid, Spain.*

Respiratory tract infections by viruses and bacteria are a leading cause of morbidity worldwide. MV130 is a mucosal vaccine that consists of whole inactivated bacteria that are common pathogens in the human respiratory tract. Previous data show that administration of MV130 reduces the frequency and severity of infectious episodes in patients with recurrent respiratory tract infections, including episodes of virus-induced wheezing in children, a significant risk factor for asthma in later life. However, the mechanisms by which MV130 provides protection against these episodes are still poorly understood. We hypothesized that the mechanism of action of MV130 may rely on trained immunity, a process by which innate immune cells challenged with certain stimuli undergo long-lasting changes that result in improved response to second related or unrelated challenge. We found that pretreatment of mouse bone-marrow derived macrophages with MV130 led to increased glycolysis resulting in lactate production maintained along several days of culture and increased TNF production following LPS challenge one week later, which are hallmarks of immune training. Moreover, pretreatment of mice with MV130 protected against an unrelated fungal systemic infection by *Candida albicans*. We further explored the potential cross-protection to viral infections and found that pretreatment with MV130 one week before infection with either vaccinia or influenza virus reduced morbidity and mortality. In summary, our data suggest that the mucosal vaccine MV130 confers cross-protection to fungal or viral infections by immune training.

WS.D3.02.04

Liver-resident memory CD8 T cells develop during chronic viral infection, have reduced effector functions but can be reactivated to clear persistent viral infection of hepatocytes

M. Bosch, *K. Manske, S. Donakonda, D. Wohlleber, P. A. Knolle, N. Kallin;*
Institute of Molecular Immunology, Munich, Germany.

Introduction: Hepatic tissue-resident memory (T_{RM}) cells are found after acute and self-limited viral infections. It is unclear whether liver-T_{RM} are also found during chronic infections. iMATEs (intrahepatic-myeloid-cell-aggregates-associated-with-T-cell-expansion) that serve as hubs for T cell-expansion to promote T-cell immunity. We characterized the impact of chronic-infection and iMATE-induction for liver-T_{RM} function and viral infection-control.
Methods:Infection with liver-targeting adenoviruses encoding ovalbumin under CMV-promoter (Ad-CMV-GOL) or hepatocyte-specific TTR-promoter (Ad-TTR-GOL) caused acute self-limited or persistent liver-infection, respectively. 100 CD45-1$^+$ Ovalbumin-specific OT-I CD8 T-cells were transferred to follow antigen-specific T-cells after infection. TLR9-ligand was injected for iMATE-induction and T-cell expansion, increased T-cell functionality and infection-control were determined by flow-cytometry and *in vivo* bioluminescence-measurement.
Results: CXCR6$^+$ liver-T_{RM} developed after self-limited Ad-CMV-GOL but also after chronic Ad-TTR-GOL liver infection and were not clonally deleted. Compared to self-limited infection, no CX_3CR1-expressing antigen-specific hepatic T cells were detected during chronic Ad-TTR-GOL-infection. At 90d after chronic Ad-TTR-GOL infection, liver-T_{RM} showed severely impaired cytotoxic-capacity and cytokine-production, thus revealing functional adaption to persistent infection. RNA-sequencing of liver-T_{RM} from acute-self-limited compared to chronic-infection and bioinformatics-analysis revealed novel molecular markers to identify exhausted liver-T_{RM} and found a single exhaustion-specific transcription factor. Importantly, iMATE-induction led to increased effector function of hepatic virus-specific T-cells and control of persistent infection.
Conclusions: Exhausted liver-T_{RM} are formed during chronic viral-infection and are identified using novel biomarkers. iMATE induction overcomes liver-T_{RM} dysfunction to control chronic infection. Thus, our results provide novel markers for immunomonitoring of patients with chronic viral hepatitis and demonstrate functional plasticity of exhausted liver-T_{RM} local hepatic stimulation within iMATEs.

WS.D3.02.05

Harnessing T-helper (Th) cell responses induced by licensed vaccines for HIV vaccine development

H. Elsayed[1,2], *G. Nabi*[3], *W. J. Mckinstry*[4], *M. Tenbusch*[1], *V. Temchura*[1], *K. Überla*[1];
[1]*University Hospital Erlangen, Institute of Clinical and Molecular Virology, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany,* [2]*National Research Center, Genetic Engineering and Biotechnology Division, Department of Microbial Biotechnology, Giza, Egypt,* [3]*Department of Molecular and Medical Virology, Ruhr-University Bochum, Bochum, Germany,* [4]*CSIRO Materials Science and Engineering, Parkville, Victoria, Australia.*

Strong T-helper responses could significantly improve antibody-based effector mechanisms induced by HIV vaccines. We recently reported that Gag-specific Th responses induced by gene-based vaccines improved the quality of the Env-specific antibody in mice after booster immunizations with virus-like particle (VLP) containing Gag and Env via intrastructural help (ISH). We now show, that immunization with pICLC-adjuvanted Gag protein also induced T helper cell responses sufficient to provide ISH and to significantly enhance Env-specific IgG2a levels after VLP booster immunizations. We therefore evaluated whether T helper cells induced by the licensed Tetanol®pur or HBVAXPro vaccines could be harnessed to improve the HIV-1 Env-specific antibody response. To this end, we generated HIV-VLPs incorporating Th epitopes for tetanus-toxoid (TT) or HBsAg (HB) within Gag. After immunizing Tetanol®pur-primed mice with VLPs containing the TT-Gag, Env-specific IgG1 antibody levels increased up to 100-fold compared to non-primed mice. The avidity of the HIV-Env antibodies was also higher. HBVAXPro-primed mice boosted with VLPs containing HB-Gag showed an approximately 10-fold increase in Env-specific IgG1 levels. Priming mice with DNA vaccines encoding TT, HBsAg or Gag predominantly improved Env-specific IgG2a responses after booster immunization with epitope-matched VLPs. However, if VLPs lacked matched epitopes, the priming immunization had no effect on the HIV-1 Env-specific antibody response. Thus, harnessing T helper cells induced by licensed vaccine to provide intrastructural help for HIV Env-specific B cells is an interesting strategy to enhance overall levels of HIV Env antibodies, to modulate their IgG subtype ratio, and to increase their avidity.

WS.D3.02.06

Design of TLR2 ligand-synthetic long peptide conjugates for therapeutic vaccination of chronic HBV patients

Y. Dou[1], ***D. T. S. Jansen***[1], *A. van den Bosch*[1], *R. A. de Man*[1], *N. van Montfoort*[1], *G. G. Zom*[2], *W. Krebber*[2], *C. J. Melief*[2], *A. M. Woltman*[1], *S. I. Buschow*[1];
[1]*Department of Gastroenterology and Hepatology, Erasmus Medical Center Rotterdam, Rotterdam, Netherlands,* [2]*ISA Pharmaceuticals BV, Leiden, Netherlands.*

Synthetic long peptide (SLP) vaccination is a promising new treatment strategy for patients with a chronic hepatitis B virus (HBV) infection. Previously, we have shown that a prototype HBV-core protein derived SLP is capable of boosting CD4+ and CD8+ T cell responses in patients *ex vivo*. These T cell responses were further enhanced by adjuvants like TLR 2 ligands. For optimal effect of a therapeutic vaccine adjuvants can be conjugated to the SLP to ensure uptake by the same cell and thus presentation of SLP-contained epitopes by matured dendritic cells (DC) only. Here, we investigated the efficacy of TLR2-ligand conjugated SLPs of different lengths and by different conjugation strategies. Engineered HBV-specific CD8+ T cells were used to analyze the cross-presentation of conjugates by *in vitro*-generated and naturally-occurring DC subsets. Results indicated better T cell responses were induced by a shorter (16 amino acids) SLP conjugate compared to longer (26 and 37 amino acids) conjugates indicating size may hamper cross presentation.

Longer SLPs however, are preferred, as inclusion of multiple CD4 and CD8 epitopes is required to ensure population-wide and long-lasting responses. To reduce size effect, we designed a conjugate in which a cathepsin-sensitive linker was placed between the TLR2 ligand and the long SLP, to facilitate endosomal release. We found this linker improved cross presentation, affected SLP intracellular processing and overall more effectively triggered patients' T cell responses *ex vivo*. These results provide an import step forward in the design of a therapeutic SLP-based vaccine to cure chronic HBV.

WS.D4.01 Protective mechanisms for microbial pathogens

WS.D4.01.01

Different composition and functional properties of splenic T cells in experimental cerebral malaria-susceptible C57BL/6 wildtype and protected *Ifnar1*$^{-/-}$ mice

J. J. Reichwald[1], *P. J. Korir*[1], *L. Jenster*[1], *A. Mueller*[2], *A. Hoerauf*[1,3], *B. Schumak*[1];
[1]*Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany,* [2]*Centre for Infectious Diseases, Parasitology Unit, Heidelberg University Hospital, Heidelberg, Germany,* [3]*German Centre for Infection Research (DZIF), partner site Bonn-Cologne, Germany.*

Introduction: Cerebral malaria (CM) is a severe complication of *Plasmodium* infection and can be studied experimentally in *Plasmodium berghei* ANKA (PbA)-infected C57BL/6 wildtype (WT) mice that develop experimental CM (ECM). PbA-infected interferon (IFN) alpha receptor knock out (*Ifnar1*$^{-/-}$) mice are protected from ECM. We characterized the composition and activity of splenic T cells between ECM-susceptible WT mice and ECM-protected mice and analysed whether these cells have different functional properties.
Material and Methods: C57BL/6 WT and *Ifnar1*$^{-/-}$ mice were infected with PbA. 6 days post infection (dpi) splenocytes were analysed by flow cytometry, cytokine/chemokine levels were determined in supernatants of splenocyte cultures with enzyme-linked immunosorbent assay (ELISA) and with intracellular FACS.
Results: ECM-positive WT mice contained on dpi 6 CD8$^+$ T cells in the brain while presenting decreased numbers of splenic CD8$^+$ T effector cells and memory cells. PbA-infected ECM-protected ko mice lacked T cell infiltration in their brains but presented higher numbers of splenic CD8$^+$ effector T cells and CD4$^+$ effector memory T cells. Whereas IFN-γ levels were comparable in splenocyte cultures of both mouse groups, WT mice contained less CCL5 in their spleens upon infection than protected mice. T cells of all PbA-groups produced more granzyme B and CCL5 than naïve controls.
Conclusions: CD8$^+$ T cells and memory cells were generally retained in the spleen of PbA-infected ECM-protected mice and might miss an egress signal migrating to the brain.
Funding: Jürgen-Manchot-Stiftung (PhD scholarship J.J.R.), BONFOR (B.S.), EXC1023 (A.H., B.S., J.J.R.)

WS.D4.01.02

A distinct subset of human CD56$^+$ cells present at baseline in HbAS-SCT-children is associated with a lower parasite density during an episode of clinical malaria

C. Loiseau[1,2], *O. K. Doumbo*[3], *B. Traore*[3], *J. L. Brady*[1,2], *C. Proietti*[2], *P. D. Crompton*[4], *D. L. Doolan*[1,2];
[1]*Australian Institute of Tropical Health and Medecine, Cairns, Australia,* [2]*James Cook University, Cairns, Australia,* [3]*University of Sciences, Technique, and Technology of Bamako, Bamako, Mali,* [4]*National Institutes of Health, Rockville, United States.*

Despite mounting substantial immune response, humans fail to control *Plasmodium* spp. infection. However, some people display better immune responses and parasite control; among them are individuals presenting the sickle-cell trait phenotype which corresponds to the heterozygous state of haemoglobin A and S (HbAS-SCT). Here, we compared the immune profiles of HbAA- and HbAS-SCT-Malian children from a malaria-endemic area, to define immunological differences that could potentially explain the relative protection observed in HbAS-SCT-patients.
Blood samples were obtained from HbAS-SCT- and HbAA-children at their uninfected baseline and during their first febrile malaria episode of the ensuing malaria season. PBMCs were isolated, activated and multicolor flow cytometry analysis were performed. Functional assays were performed on PBMCs from healthy donors.
At baseline, the frequency of CD56$^+$ cells was increased in HbAS-SCT-children *(P<0.05)*; and was inversely correlated with parasite density *(r=-0.76, P<0.05)*. The CD38^{high} subset in CD56$^+$ cells was reduced in HbAS-SCT-children *(P<0.01)*; and was correlated with parasite density *(r=-0.90, P<0.01)*. Thus, the increased frequency of the CD56$^+$ cells in CD3$^-$ lymphocytes of HbAS-SCT-children appeared to be due to their CD38$^{low/med}$ subset. This subset of NK cells was inversely correlated with parasite density *(r=-0.86, P<0.05),* characterized by an increased expression of HLA-DR and CD45RO (*P<0.001*) and a 15-fold increased production of IFN-γ compared to the CD38^{high} subset.
The CD38$^{low/med}$ subset represents a novel population of CD56$^+$ cells identified at baseline in HbAS-SCT-children and is potentially associated with enhanced parasite control. The HLA-DR$^+$CD45RO$^+$CD38$^{low/med}$ CD56$^+$CD3$^-$ cells may represent a potential predictive signature of immunity to malaria.

WS.D4.01.03

Transcription factor T-bet plays a complex role in B cell mediated immune response against *Plasmodium* infection

M. Akkaya, *P. W. Sheehan, C. K. Cimperman, B. P. Theall, M. Pena, S. K. Pierce;*
National Institutes of Health, Rockville, United States.

Malaria is a global health concern which affects over 200 million individuals worldwide. Although immune system rapidly responds to *Plasmodium* infection with specific antibodies, natural antibodies fail to establish long term protection, often leading to repetitive infections and chronicity. Recent studies showed that in chronic malaria setting both B cells and T cells induce T-bet transcription factor expression. Yet it is not completely understood whether this is a host defense strategy or a result of disease pathogenesis. Here using transgenic mouse models, we show that deficiency of T-bet reduces survival of mice upon infection with *Plasmodium chabaudi*, suggesting a protective role. Similarly, using Tbet-zsgreen transgenic mice we showed that conditions specific to *P.chabaudi* infection drive T-bet expression in multiple B cell subsets in a time dependent fashion. However, no such induction is shown when mice are immunized with protein antigens adjuvanted with Alum. On the other hand, bone marrow chimeric animals reconstituted with equal amounts of congenically labeled WT and T-bet KO bone marrow cells showed that for most B cell subsets the lack of T-bet does not affect WT and KO cell ratio upon *Plasmodium* infection indicating that T-bet may not be equally crucial for all B cell responses to infection. However in vitro stimulation of WT and T-bet KO B cells with Anti-IgM plus IFN-gamma reveals that expression of several B cell markers depend at least in part on the presence of T-bet. These findings suggest a complex role of T-bet in the development of humoral responses.

WS.D4.01.04

Within host-adaptation of *Bordetella pertussis* under vaccine immune pressure

E. van Schuppen[1,2], *A. Zeddeman*[3,1,2], *K. E. Kok*[3], *M. van Gent*[3], *K. J. Heuvelman*[3], *M. J. Bart*[3], *H. G. van der Heide*[3], *F. J. van Opzeeland*[1,2], *S. van Selm*[1,2], *M. I. de Jonge*[1,2], *R. de Groot*[1,2], *F. R. Mooi*[3,1,2], *D. A. Diavatopoulos*[1,2];
[1]*Section Pediatric Infectious Diseases, Laboratory of Medical Immunology, Radboud Institute for Molecular Life Sciences, Radboudumc, Nijmegen, Netherlands,* [2]*Radboud Center for Infectious Diseases, Radboudumc, Nijmegen, Netherlands,* [3]*Centre for Infectious Diseases Research, Diagnostics and Screening (IDS), National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands.*

The first vaccines against pertussis were introduced in the 1940s-1960s and were comprised of killed bacteria (wP vaccines). Despite the high effectiveness of wPs, high reactogenicity led to their replacement with acellular pertussis vaccines (aPs). aPs contain several *B. pertussis* antigens, including pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (Prn), and fimbriae (Fim). We investigated the efficacy of various aP and wP vaccines against infection with modern Prn-expressing and Prn-deficient strains.
Naïve and vaccinated mice were challenged with Prn-producing (Prn$^+$) and Prn-deficient (Prn$^-$) strains and bacterial load was determined in the nose and lungs. Phase variation was studied using a Ligase Detection Reaction. FHA expression was determined by Luminex and Western blot. Antibody deposition on *B. pertussis* was studied by flow cytometry.
We show essential differences in protection against Prn$^+$ and Prn$^-$ strains in relation to the type and composition of pertussis vaccines, as well as the location in the airways. We describe the emergence of bacteria in the lower respiratory tract that have switched-off FHA expression due to phase variation in the *fhaB* gene. These mutants were positively selected for in Prn$^-$ strains following vaccination with aP but not wP vaccines. Of concern, Prn$^-$ FHA$^-$ strains are not recognized anymore by aP vaccine-induced opsonizing antibodies.
This study demonstrates that in addition to the loss of Prn as a protective antigen, *B. pertussis* strains can also switch-off FHA expression, suggesting that immunity to two of the most widely used pertussis vaccine antigens may be compromised.

WS.D4.01.05

Transphagocytosis, a novel mechanism for bacterial uptake by B cells

R. García Ferreras[1,2], *G. Ramírez Santiago*[1,2], *A. Cruz Adalia*[1,2], *J. Osuna Pérez*[1,2], *M. Torres Torresano*[1,2], *Y. R. Carrasco*[1], *E. Veiga Chacón*[1,2];
[1]*Centro Nacional de Biotecnología (CNB), Madrid, Spain,* [2]*CSIC, Madrid, Spain.*

It is now well established that B cells are antigen presenting cells (APC). Indeed, it is known that, *in vivo*, B cells are able to uptake soluble and surface-tethered antigens presented on different types of cells. However, the way of B cells to capture bacteria remains unknown. Interestingly, we have shown that B cells capture bacteria from previously infected dendritic cells (DC) during DC - B cells closed contact, a process called transphagocytosis. Uptaken bacteria were rapidly degraded by B cells. Bacterial antigens resulting from degradation were cross-presented to CD8+ T which resulted strongly activated and proliferated. Taking into account that only very few cells are able to activate naive CD8+ T cells triggering its proliferation, we propose transphagocytosis as a potent mechanism to generate a cytotoxic response. Therefore, we characterize that a previous exposition of Pathogen Associated Molecular Patterns (PAMPS) might improve these immune abilities by B cells against infection.

WS.D4.01.06

Human vaginal and skin dendritic cell subsets control ZIKA virus infection

***J. Eder**, T. B. Geijtenbeek;*
Academic Medical Center Amsterdam (AMC), Amsterdam Infection and Immunity Institute, Amsterdam, Netherlands.

ZIKA virus (ZIKV) is a member of the Flaviviridae family and recent outbreaks have shown that it is very infectious and infection has been associated with abnormal fetal brain development. ZIKV is transmitted through mosquito bites and sexual contact. However, little is known about first cell targets and subsequent viral dissemination as well as immune responses induced by infection. Here we investigated the role of dendritic cell (DC) subsets in ZIKV infection of human skin and vaginal mucosa, initial tissues involved in infection. Therefore, human primary DCs were isolated from skin, buffy coats and vaginal mucosa and infected with a wild-type ZIKV strain. Moreover, we used an ex-vivo infection model from skin and also embryonic neuronal cells as target cells during the different assays. Notably, mucosal and epidermal Langerhans cells did not become infected by ZIKV, whereas in contrast ZIKV efficiently infected human submucosal DCs. Infected DCs transmitted the virus to target cells, suggesting that DCs are involved in ZIKV dissemination. ZIKV infection of DCs induced a strong type I Interferon (IFN) response, DC maturation and expression of pro-inflammatory cytokines. The type I IFN response limits ZIKV infection as inhibition of IFN receptors enhanced ZIKV infection. These data strongly suggest that submucosal DCs are one of the first target cells for ZIKV and are involved in induction of innate and adaptive immunity to the virus. Dissecting the adaptive immune responses as well as viral dissemination is important to develop strategies to prevent ZIKV-associated pathologies.
European Research Council, Advanced grant (670424).

WS.D4.02 Responses to mucosal microbial pathogens

WS.D4.02.01

Combined chemical genetics and data-driven bioinformatics approach identifies receptor tyrosine kinase inhibitors as host-directed drugs for intracellular bacterial infections

*C. J. Korbee[1], M. T. Heemskerk[1], D. Kocev[2], E. van Strijen[1], O. Rabiee[1], K. L. Franken[1], L. Wilson[1], N. D. Savage[1], S. Džeroski[2], T. H. Ottenhoff[1], **M. C. Haks**[1];*
[1]LUMC, Leiden, Netherlands, [2]Josef Stefan Institute, Ljubljana, Slovenia.

Antibiotic-resistance poses rapidly increasing global problems in combatting multidrug-resistant (MDR) infectious diseases like MDR tuberculosis, prompting for novel approaches including host-directed therapies (HDT). Intracellular pathogens like *Salmonellae* and *Mycobacterium tuberculosis* (*Mtb*) exploit host signaling pathways to survive intracellularly. Thus far, only very few HDT-compounds targeting host pathways have been identified for HDT. In a Library Of Pharmacologically Active Compounds (LOPAC)-based drug-repurposing screen, using a novel intracellular infection screening assay, we have identified multiple compounds, which target Receptor Tyrosine Kinases (RTKs) and inhibited intracellular *Mtb* and *Salmonellae* more potently than currently known HDT-compounds. By developing a data-driven *in silico* model based on confirmed targets from public databases, we successfully predicted additional efficacious HDT-compounds. These also appeared to target host RTK signaling and inhibited intracellular *Mtb* including MDR-*Mtb*. A complementary human kinome siRNA screen independently confirmed the role of RTK signaling and kinases (BLK, ABL1 and NTRK1) in host control of *Mtb*. These three approaches validate RTK signaling as a new drugable host pathway for HDT against intracellular bacteria (Nature Communications, 2018).
Grant support: STW (Grant no. 13259), ZonMw-TOP (Grant no. 91214038), 7th Framework Programme PHAGOSYS (HEALTH-F4-2008-223451).

WS.D4.02.02

Host-directed, autophagy-modulating compounds restrict intracellular *Mycobacterium tuberculosis* and *Salmonella* Typhimurium infection in human macrophages

*M. T. Heemskerk, C. J. Korbee, J. Esselink, C. Carvalho dos Santos, F. Vrieling, I. Gordijn, L. Wilson, T. H. Ottenhoff, **M. C. Haks**;*
LUMC, Leiden, Netherlands.

The persistent increase of multidrug-resistant (MDR) *Mycobacterium tuberculosis* (*Mtb*) infections negatively impacts tuberculosis (TB) treatment outcomes, prompting for the development of alternative strategies in addition to conventional antibiotics. Host-directed therapies (HDT) pose such an alternative, especially since the success of *Mtb* can partly be explained by its successful manipulation of host signalling pathways in order to create a pathogen favourable intracellular environment. Similarly, *Salmonella* infections, causing a.o. typhoid fever, are accompanied by significant mortality and pose therapeutic difficulties because also this pathogen modifies host regulatory networks to ensure its intracellular survival. One key mechanism of host defence is autophagy, which is of particular interest for HDT given its important and versatile functions in TB pathogenesis. Here a commercially available library of compounds with proven and defined autophagy-modulating activity was screened for small molecules capable of inhibiting intracellular (but not extracellular) *Mtb* and *Salmonella* Typhimurium *(Stm)* survival.
Four FDA-approved compounds were found to act against (MDR-)*Mtb* and *Stm* in primary human macrophages in a host-directed manner, and displayed synergy with suboptimal doses of Rifampicin. To elucidate their mechanisms of action various functional assays were performed including monitoring of autophagy, lysosomal activity, and reactive oxygen species (ROS) generation. The results showed clear differences in the mechanisms targeted by each of the compounds. Current efforts, including studies in a zebrafish infection model, are directed to further dissect the mechanisms by which these autophagy-modulators decrease intracellular bacterial survival.
Grant support: Netherlands Organisation for Scientific Research-TTW (Grant no. 13259), ZonMw-TOP (Grant no. 91214038).

WS.D4.02.03

The Mal-IFN-γ receptor axis receptor axis and TIRAP-S180L polymorphism as determinants of susceptibility to pneumococcal disease

***N. Munoz-Wolf**[1], C. M. Ni Cheallaigh[2], M. Herrlein[3], D. Golenbock[4], K. A. Fitzgerald[4], L. A. O'Neill[1], E. C. Lavelle[1];*
[1]School of Biochemistry and Immunology, Trinity Biomedical Science Institute -Trinity College Dublin, Dublin, Ireland, [2]2Institute of Molecular Medicine, Trinity College Dublin & St. James's Hospital – Ireland., Dublin, Ireland, [3]Goethe University Frankfurt/Main, Frankfurt, Germany, [4]University of Massachusetts Medical School, Division of Infectious Disease & Immunology, Massachusetts, United States.

Genetic single-nucleotide polymorphisms (SNP) affecting immune receptors or their signalling components can influence susceptibility to infections. TIRAP encodes the MyD88-adaptor like protein (Mal), a bridging adaptor for TLR4 and TLR2. A SNP in TIRAP resulting in the substitution S180L alters susceptibility to invasive pneumococcal infection, tuberculosis and sepsis. $TIRAP^{L/L}$ homozygosity increases susceptibility, while heterozygotes $TIRAP^{S/L}$ are protected compared to $TIRAP^{S/S}$ "wild-types". We discovered a TLR-independent role for Mal in tuberculosis whereby it mediates interferon (IFN)-γ receptor (IFNGR) signalling which is affected in $TIRAP^{L/L}$ impairing autophagy and mycobacterial killing in macrophages. Here we investigated the role of Mal and the Mal-IFNGR axis in invasive pneumonia caused by *Streptococcus pneumoniae,* an important human pathogen that kills >1.6 million people/year.
We showed that Mal is required for protection against pneumococci, as evidenced by the hyper-susceptibility of $Tirap^{-/-}$ mice to invasive pneumonia. Despite the involvement of Mal in TLR2 and TLR4 signalling, $Tlr2/4^{DKO}$ immortalised macrophages (iBMMs) expressed comparable *Tnfa* mRNA to wild-types when infected with pneumococci or stimulated with TLR ligands, other than LPS and Pam3CSK. However, $Tirap^{-/-}$ and in particular $Tirap^{L/L}$ over-expressed *Tnfa* and *Il6* mRNA in response to all stimuli, a response that was exacerbated by pre-treatment with rIFN-γ. Besides, $Tirap^{-/-}$ and $Tirap^{L/L}$ were less efficient at killing pneumococci *in vitro* compared to $Tlr2/4^{DKO}$ and wild-types. Finally, infected $Tirap^{-/-}$ mice had increased alveolar infiltration of inflammatory monocytes. These results suggest a novel TLR-independent role for Mal during pneumonia highlighting its key role as a modulator of IFN-γ-induced inflammation in the lungs.

WS.D4.02.04

Dual RNA-seq unveils novel host-pathogen interactions during colonic bacterial infections

***F. Wan**;*
Johns Hopkins University, Baltimore, United States.

Attaching/Effacing (A/E) pathogens including enteropathogenic *Escherichia coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and the rodent equivalent *Citrobacter rodentium* (CR) are important causative agents of foodborne diseases. A/E pathogen infections cause severe morbidity and mortality in immunocompromised hosts with low interleukin-22 (IL-22); however the crucial host-pathogen interactions and the pivotal A/E virulence proteins (effectors) under immunocompromised conditions, remain elusive. We utilize a "dual RNA-sequencing" approach to simultaneously profile gene expression in the pathogen and the host, and identify an extracellular metalloprotease as a novel virulence factor during *C. rodentium* infection in the immunocompromised $Il22^{-/-}$ mice. Genetic deletion of the extracellular protease substantially attenuates *C. rodentium* infection-induced morbidity and mortality in $Il22^{-/-}$ mice, which underscores the pathophysiological relevance of bacterial extracellular proteases. Moreover, the extracellular protease deficiency impedes the inflammatory cytokine gene expression in the *C. rodentium*-infected $Il22^{-/-}$ colon. These findings reveal novel host-pathogen interactions during *C. rodentium* infection in $Il22^{-/-}$ mice, which could provide novel strategies to control A/E pathogen infections under low IL-22 immunocompromised conditions associated with chronic HIV infection, organ transplantation, and other diseases.
Supported in part by NIH Grant GM111682.

WS.D4.02.05
iNOS- AND NOX1- DEPENDENT ROS PRODUCTION MAINTAINS BACTERIAL HOMEOSTASIS IN THE ILEUM OF MICE

A. Kielland[1], *C. Matziouridou*[1], *S. Rocha*[1], *K. Rudi*[1], *O. A. Haabeth*[2], *H. Carlsen*[1];
[1]*Norwegian University of Life Sciences (NMBU), Ås, Norway,* [2]*University of Oslo, Oslo, Norway.*

The intestinal epithelium constitute a first line of defense against gut microbes, which includes secretion of various antimicrobial substances. Reactive oxygen species (ROS) are well characterized as part of the innate phagocytic immunity; however, a role in controlling microorganisms in the gut lumen is less clear.
Here, we demonstrate, through *in vivo* imaging, a remarkably high production of ROS in the ileum of normal healthy mice. The ROS production depends on the enzymes iNOS (NO producer) and NOX1 (superoxide producer). NO and superoxide rapidly combine to form peroxynitrite. Peroxynitrite is bactericidal and one of the important ROS in respiratory burst. Mice deficient in iNOS and NOX1 have increased bacterial load and a significant shift in the microbiota composition of the ileum. Furthermore, the ROS appear to prevent reflux of microbiota from large intestine and be of relevance for bacterial overgrowth and translocation.
Our data suggests a new role of ROS in regulating the bacterial content at the border of the small and large intestine. This may imply a unique role of ileum in maintaining homeostasis of gut microbes through production of peroxynitrite with potential importance for preventing reflux from the large intestine, bacterial overgrowth and translocation. Furthermore, as several conditions and diseases such as Crohn's disease and SIBO have been linked to impaired microbial homeostasis in ileum, these findings might provide new insights into pathogenesis related mechanisms that can indicate strategies for disease prevention.

WS.D4.02.06
NLRP11 negatively regulates NF-κB and type I interferon responses

K. Ellwanger[1], *E. Becker*[1], ***I. Kienes***[1], *A. Sowa*[1], *Y. Postma*[1], *Y. Cardona Gloria*[2], *A. N. Weber*[2], *T. A. Kufer*[1];
[1]*University of Hohenheim, Stuttgart, Germany,* [2]*University of Tübingen, Tübingen, Germany.*

Nucleotide-binding domain and leucine-rich repeat containing proteins (NLRs) are a group of intracellular pattern recognition receptors, involved in the regulation and induction of innate and adaptive immunity in mammals. However, the function of about half of the currently identified NLRs remains elusive. Here we provide a functional characterization of NLRP11, a primate-specific member of the NLR family. We show that NLRP11 is highly expressed in reproductive tissue and in immune cells, including myeloid cells, B cells and some B cell lymphoma lines. Overexpression of NLRP11 in human cells did not trigger key innate immune signalling pathways including NF-κB, type I interferon and caspase-1. By contrast, expression of NLRP11 repressed MyD88-induced NF-κB and TBK1-induced type I interferon responses. This effect was independent of the PYD domain and ATPase activity of NLRP11 but mediated by its LRR domain. Accordingly, knock-down of NLRP11 in human myeloid THP1 cells enhanced lipopolysaccharide (LPS) and Sendai Virus (SeV)-induced cytokine and interferon responses upon NLRP11 depletion. In summary, our work identifies a novel role of NLRP11 in the regulation of inflammatory responses in human cells.

WS.D4.03 Immune sensing of microbial infections

WS.D4.03.01
Functional cooperation between complement factor H and the long pentraxin PTX3 in the immune response to *Aspergillus fumigatus*

R. Parente[1], *F. Petroni*[1], *M. Stravalaci*[1,2], *M. Sironi*[1], *R. Leone*[1], *S. Valentino*[1], *A. J. Day*[3], *B. Bottazzi*[1], *A. Mantovani*[1,2], ***A. Inforzato***[1,2];
[1]*Humanitas Clinical and Research Center, Rozzano, Italy,* [2]*Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Italy,* [3]*Wellcome Trust Centre for Cell-Matrix Research, Division of Cell-Matrix Biology and Regenerative Medicine, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, United Kingdom.*

Aspergillus fumigatus (AF) is the major etiologic agent of Invasive Aspergillosis (IA), a severe infection amongst immunocompromised individuals. A pivotal role in the host resistance to this pathogen is played by polymorphonuclear neutrophils (PMNs) and complement. The long pentraxin PTX3 exerts opsonic activity towards AF conidia, and enhances their phagocytosis and killing by PMNs via complement pathways. Here we characterized the molecular crosstalk between PTX3 and complement in the opsono-phagocytosis of AF. Complement activation on AF conidia was assessed by Western Blotting and ELISA using complement proteins and human sera depleted of selected complement components in the presence and absence of recombinant PTX3. In parallel experiments, AF phagocytosis and killing by human and murine PMNs was assessed *in vitro* by Flow Cytometry. We found that PTX3 promotes the selective recruitment of C3b on the conidial wall, by targeting the alternative pathway (AP) of complement. To our surprise, factor H (main inhibitor of AP) was necessary for this activity, thus pointing to a novel function (activating rather than inhibitory) of this factor when combined with PTX3. Consistent with this, factor H and complement receptor 1 (CR1, major receptor of C3b) were required for the pro-phagocytic and pro-killing properties of PTX3. Therefore, a cooperation was observed between factor H and PTX3 with an unexpected functional outcome: enhanced recruitment of C3b on AF. Given the potent opsonic activity of C3b (via CR1), we believe that this is a major mechanism of PTX3 in the promotion of AF phagocytosis and killing by PMNs.

WS.D4.03.02
Orchestration of systemic anti-fungal Th17 immunity and immune pathology by a single member of the mycobiome

P. Bacher[1], *T. Hohnstein*[1], *E. Beerbaum*[1], *M. Röcker*[2], *S. Kaufmann*[1], *C. Brandt*[1], *J. Röhmel*[1], *U. Stervbo*[3], *M. Nienen*[3], *N. Babel*[3], *J. Milleck*[4], *M. Assenmacher*[4], *O. A. Cornely*[5], *G. Heine*[1], *M. Worm*[1], *P. Creutz*[1], *C. Ruwwe-Glösenkamp*[1], *L. E. Sander*[1], *O. Kniemeyer*[2], *A. A. Brakhage*[2], *C. Schwarz*[1], *A. Scheffold*[6];
[1]*Charité-Universitätsmedizin Berlin, Berlin, Germany,* [2]*Hans Knoell Institute (HKI) Jena and Friedrich Schiller University Jena, Jena, Germany,* [3]*Ruhr University Bochum, Bochum, Germany,* [4]*Miltenyi Biotec GmbH, Bergisch Gladbach, Germany,* [5]*University Hospital of Cologne, Cologne, Germany,* [6]*Christian-Albrechts University Kiel, Kiel, Germany.*

Introduction: Th17 cells protect against bacteria and fungi, but also contribute to chronic inflammation. In humans, Th17 responses are particularly important against *Candida albicans,* a mucocutaneous fungal pathobiont. In contrast, the role of Th17 cells for other pathogenic fungal species is unclear. Pulmonary fungus-related disorders and sensitizations are often associated with chronic respiratory diseases such as asthma, COPD and cystic fibrosis. Also these patients show increased Th17 cytokine levels, which correlate with disease severity. However, the capacity of most fungal species to induce human Th17 responses and their potential contribution to pulmonary diseases is currently unclear.
Methods: We used antigen-reactive T cell enrichment (ARTE) for the *ex vivo* analysis of human T helper cell responses against 25 common human pathogenic fungal species.
Results: We show that *C. albicans* is the sole direct fungal inducer of Th17 cells in humans. For all other fungi tested, minor and variable fractions of Th17 cells were detected. Surprisingly, these Th17 cells, but not Th1 cells against the same fungal species, were strongly and selectively cross-reactive against *C. albicans*. Patients with pulmonary inflammation displayed elevated frequencies of cross-reactive *A.fumigatus* Th17 cells, suggesting their specific contribution to lung pathology. In particular in patients with allergic sensitization to *A.fumigatus,* increased Th17 responses strongly correlated with acute ABPA.
Conclusions: Our data identify *C. albicans* as the major fungal inducer of human Th17 responses. We provide a unique example how protective Th17 immunity may simultaneously promote immune pathology when deviated to different target antigens and tissues via heterologous immunity.

WS.D4.03.04
Inherited p40^{phox} deficiency differs from chronic granulomatous disease: Distinctive molecular, cellular and clinical phenotypes

A. van de Geer[1], *A. Nieto-Patlán*[2], *D. Roos*[1], *J. Casanova*[3], *T. W. Kuijpers*[4], *J. Bustamante*[2];
[1]*Sanquin, Amsterdam, Netherlands,* [2]*Laboratory of Human Genetics of Infectious Diseases, Necker Branch, INSERM U1163, Necker Hospital for Sick Children, Paris, France,* [3]*St Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, New York, United States,* [4]*Emma Children's Hospital, Amsterdam, Netherlands.*

Chronic granulomatous disease (CGD) is a well-defined recessive primary immunodeficiency caused by loss-of-function mutations in any of the genes encoding four phagocyte NADPH oxidase subunits (p22^{phox}, p47^{phox}, p67^{phox}, and gp91^{phox}). In contrast, loss-of-function mutations in the *NCF4* gene, encoding the p40^{phox} subunit, have only been described once. We report 24 p40^{phox}-deficient patients from 12 additional families in eight countries. These patients display eight different *NCF4* mutations, homozygous in 11 families and compound heterozygous in another. Upon over-expression in NB4 neutrophil-like cells and EBV-transformed B cells *in vitro,* the mutant alleles were loss-of-function, except for one hypomorphic allele. Particle-induced NADPH oxidase activity in the patients' neutrophils was subnormal, whereas PMA-induced DHR oxidation, a widely used test for CGD diagnosis, was sometimes normal. Moreover, NADPH oxidase activity of EBV-transformed B cells was also subnormal, whereas that of mononuclear phagocytes was normal. Finally, unlike in CGD, neutrophil killing of *Candida albicans* and *Aspergillus fumigatus* hyphae was maintained, whereas that of *Staphylococcus aureus* was impaired both in CGD and in p40^{phox} deficiency. Patients suffer from auto-inflammation and peripheral infections, but not from invasive bacterial and fungal infections. Moreover, all patients are alive at ages 1-46 years and only four of them have received hematopoietic stem cell transplantation. In conclusion, inherited p40^{phox} deficiency underlies a distinctive condition, evoking a mild form of CGD, with inflammatory lesions and peripheral infections, but without invasive bacterial and fungal disease. Its detection should not rely on the usual clinical test of PMA-induced oxidation of substrates by neutrophils.

WS.D4.03.05
TRIM5α-mediated autophagy: Where HIV-1 restriction and molecular inflammation meet

A. P. Cloherty, J. L. van Hamme, R. Sarrami-Forooshani, N. A. Kootstra, T. B. Geijtenbeek, ***C. M. S. Ribeiro****;*
University of Amsterdam, Amsterdam, Netherlands.

Contemporary HIV-1 antiretroviral therapy is highly effective, however, treated HIV-1 patients suffer from severe comorbidities due to persistent viral replication and chronic inflammation. There is thus an urgent need to identify therapeutic targets to enhance antiviral immunity and control inflammation in treated HIV-1 patients.
Autophagy functions as an antiviral defense mechanism by degrading viruses and instructing adaptive T-cell responses. We were the first to establish that human TRIM5α mediates assembly of an autophagy-activating scaffold to HIV-1 components, which targets HIV-1 for autophagic degradation and restricts infection of a human dendritic cell (DC) subset. Here, we show a novel protective role for autophagy machinery in HIV-1 patients. We have recently identified a gene polymorphism in a regulator of TRIM5α-mediated autophagy that is associated with decreased plasma viral load and improved survival in HIV-1 patients from the Amsterdam Cohort Studies. This polymorphism correlates with increased autophagy levels and heightened $CD8^+$T-cell responses *in vitro*, as well as with decreased susceptibility to HIV-1 infection of emigrated DC subsets in *ex vivo* explant models. These findings underscore the pivotal role for autophagy in limiting HIV-1 replication and boosting antiviral T-cell immunity. Furthermore, our preliminary data identify a novel link between TRIM5α-mediated autophagy and virus-induced molecular inflammation. We have demonstrated that TRIM5α and ATG13 are key in the secretion of inflammatory mediators such as IL-1β and TNFα after viral infection.
Hence, our data underscore the *in vivo* relevance of autophagy mechanisms and the therapeutic potential of targeting autophagy to intervene in acute and chronic HIV-1 infections.

WS.D4.03.06
Type I interferon-dependent NETosis supports biofilm formation and survival of *Pseudomonas aeruginosa* during lung infection

E. Pylaeva*, S. Bordbari, I. Spyra, S. Lang, J. Jablonska;*
University Hospital, Ear, Nose, and Throat Department, Essen, Germany.

The enhanced predisposition to bacterial complications in cancer or viral infections is known. These clinical situations are often associated with elevated levels of type I interferons (IFN). As neutrophils are the major antibacterial responders in acute phase of infection, we set up to reveal the role of IFNs in the regulation of neutrophil bactericidal properties. In the model of acute *Pseudomonas aeruginosa* pneumonia we observed elevated bacterial load and lung tissue damage in WT mice, as compared to IFN-deficient animals. We observed enhanced neutrophil extracellular traps (NETs) release by WT lung neutrophils, accompanied by the elevated biofilm formation and survival of *P. aeruginosa*. Interestingly, infection with biofilm-negative *P. aeruginosa* revealed no differences in bacterial load between WT and IFN-deficient mice. Treatment of mice with rmIFN-beta raised biofilm formation. Disruption of NETs using DNase prevented biofilm formation. In agreement, treatment of mice with ROS scavenger N-acetylcysteine decreased NETs release, biofilm content and bacterial load in the lung, confirming the role of NETosis-dependent biofilm formation as supporter of bacterial survival. Summarizing, during *Pseudomonas* infection IFNs stimulate NETs release by neutrophils, which in turn supports biofilm formation by *Pseudomonas*. Biofilm protects bacteria from the immune system and leads to their persistence in the lung. Biofilms can also be responsible for the antibiotic-resistance typical for *Pseudomonas*, therefore targeting NETosis may help to develop effective treatment strategies for persistent infections with this pathogen.

WS.D4.04 Virus-host interactions

WS.D4.04.01
Human cytomegalovirus reprograms hematopoietic progenitor cells into immunosuppressive monocytes to achieve latency

K. Zen*;*
Nanjing University, Atlanta, China.

The precise cell type hosting latent human cytomegalovirus (HCMV) remains elusive. Here we report that HCMV reprograms human hematopoietic progenitor cells (HPCs) into a unique monocyte subset to achieve latency. Unlike conventional monocytes, this monocyte subset possesses higher levels of B7-H4, IL-10 and iNOS, longer lifespan and strong immunosuppressive capacity. Cell sorting of peripheral blood from latently infected human donors confirms that only this monocyte subset, representing less than 0.1% of peripheral mononuclear cells, is HCMV genome-positive but immediate-early (IE)-negative. Mechanistic studies demonstrate that HCMV promotes the differentiation of HPCs into this monocyte subset by activating cellular signal transducer and activator of transcription 3 (STAT3). In turn, this monocyte subset generates a high level of nitric oxide (NO) to silence HCMV IE transcription and promote viral latency. By contrast, the US28-knockout HCMV mutant, which is incapable of activating STAT3, fails to reprogram the HPCs and achieve latency. Our findings reveal that via activating STAT3/iNOS/NO axis HCMV differentiates human HPCs into a longevous, immunosuppressive monocyte subset for viral latency.

WS.D4.04.02
Killer cell proteases target viral immediate-early proteins to control cytomegalovirus infection in a noncytotoxic manner

L. Shan*[1], J. Meeldijk[1], B. Blijenberg[1], A. Hendriks[1], K. J. van Boxtel[1], S. P. van den Berg[2], A. Svrlanska[3], T. Stamminger[4], M. R. Wills[2], N. Bovenschen[1];*
[1]University Medical Center Utrecht, Utrecht, Netherlands, [2]University of Cambridge, Cambridge, United Kingdom, [3]University of Erlangen-Nuremberg, Erlangen, Germany, [4]Ulm University Medical Center, Ulm, Germany.

Human cytomegalovirus (HCMV) is the most frequent viral cause of congenital defects and can trigger devastating disease in immune-suppressed patients. Cytotoxic lymphocytes (*e.g.* $CD8^+$ T cells and NK cells) control HCMV infection by releasing the pore-forming protein perforin and five cytotoxic granzymes (GrA, GrB, GrH, GrK, and GrM) towards virus-infected cells. Perforin facilitates the cellular entry of granzymes, which are believed to kill infected host cells through cleavage of intracellular death substrates. However, it has recently been demonstrated that the *in vivo* killing capacity of cytotoxic T cells is limited. This raises the question whether cytotoxic lymphocytes can also control HCMV infection in a noncytotoxic manner. In the present study, we demonstrate that (primary) cytotoxic lymphocytes block HCMV dissemination and induce the degradation of viral immediate-early (IE) proteins IE1 and IE2 in HCMV-infected cells in a cell death-independent noncytotoxic manner at low effector:target cell ratios. Interestingly, both IE1 and/or IE2 are directly proteolyzed by all human granzymes, with GrB and GrM being most efficient. GrB and GrM cleave IE1 after Asp^{398} and Leu^{414}, respectively, resulting in IE1 dislocation, IE1 instability, and functional impairment of IE1 to interfere with the JAK-STAT signaling pathway. Furthermore, GrB and GrM cleave IE2 after Asp^{184} and Leu^{173}, respectively, resulting in IE2 dislocation and functional abolishment of IE2 to transactivate the HCMV UL112 early promoter. Taken together, our data indicate that cytotoxic lymphocytes can employ noncytotoxic ways to control HCMV infection via granzyme-mediated targeting of indispensable viral proteins during lytic infection.

WS.D4.04.03
Pathological role of anti-CD4 antibodies in HIV-infected immunologic non-responders under viral suppressive antiretroviral therapy

W. Jiang*, Z. Luo;*
Medical University of South Carolina, Charleston, United States.

Abstract
Increased mortality and morbidity occurs in human immunodeficiency virus (HIV)-infected patients who fail to increase $CD4^+$ T cell counts despite achieving viral suppression with antiretroviral therapy (ART). Here we identified an underlying mechanism. Significantly elevated plasma levels of anti-CD4 IgGs were found in HIV^+ immunologic non-responders ($CD4^+$ T cell counts ≤ 350 cells/μl) [median (interquartile range), 91.8 ng/mL (53.2-165.8)] compared to HIV^+ immunologic responders ($CD4^+$ T cell counts ≥ 500 cells/μl) [26.0 ng/mL (15.9-81.6)] and healthy controls [14.5 ng/mL (9.2-24.1)] ($P < 0.001$ between non-responders and responders or healthy controls, non-parametric Mann-Whitney test). Higher plasma level of anti-CD4 IgG correlated with blunted $CD4^+$ T cell recovery ($r = -0.53$, $P = 0.0002$, Spearman correlation test). Furthermore, purified anti-CD4 IgGs from HIV^+ immunologic non-responders induced NK cell-dependent $CD4^+$ T cell cytolysis [19.1% (5.8-50.3) vs. 0.04% (0.03-0.05) for treatment with anti-CD4 IgGs and anti-CD4-depleted IgGs respectively] and apoptosis [36.9% (26.6-44.5) vs. 15.4% (14.3-16.8) for treatment with anti-CD4 IgGs and anti-CD4-depleted IgGs respectively] through antibody-dependent cell-mediated cytotoxicity (ADCC) *in vitro*. We also found that anti-CD4 IgG-mediated ADCC exerts marginally increased apoptosis on naive relative to memory $CD4^+$ T cells ($P = 0.06$). Consistently, increased frequencies of $CD107a^+$ NK cells and profound decreases of naive $CD4^+$ T cells were observed in immunologic non-responders compared to responders and healthy controls *ex vivo*. These data indicate that autoreactive anti-CD4 IgGs may play an important role in the blunted $CD4^+$ T cell reconstitution despite effective ART.

WS.D4.04.04

HIV-1 exposure enhances sexual transmission of HCV by inducing Syndecan-4 on Langerhans cells

B. M. Nijmeijer[1], *R. Sarrami Forooshani*[1], *J. Eder*[1], *G. S. Steba*[2], *R. R. Schreurs*[1], *S. M. Koekkoek*[2], *R. Molenkamp*[2], *J. Schinkel*[2], *P. Reiss*[3,4], *M. L. Siegenbeek van Heukelom*[4,5], *M. van der Valk*[4], *C. M. Ribeiro*[1], *T. B. Geijtenbeek*[1];

[1]*Department of Experimental Immunology, Academic Medical Center, Amsterdam Infection and Immunity Institute, University of Amsterdam, Amsterdam, Netherlands,* [2]*Department of Medical Microbiology, Clinical Virology laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands,* [3]*Department of Global Health, Academic Medical Center, and Amsterdam Institute for Global Health and Development , Academic Medical Center HIV Monitoring Foundation, Amsterdam, Netherlands,* [4]*Department of Internal Medicine, Division of Infectious Diseases, Amsterdam Infection and Immunity Institute, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands,* [5]*Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands.*

The significant rise in incidence of Hepatitis C virus (HCV) infection among HIV-infected men-who-have-sex-with-men (MSM) suggests that HCV under specific circumstances is transmitted via sexual contact. The mechanisms remain unclear. Here we investigated the molecular mechanism of sexual transmission of HCV. Analyses of mucosal anal biopsies from HIV-1-infected MSM identified Langerhans cells (LCs) within mucosal tissue. However, immature LCs were neither infected nor transmitted HCV to hepatocytes *in vitro* and *ex vivo*. As sexual transmission is mostly observed within HIV-1-infected individuals, we pre-exposed tissues with HIV-1 and, strikingly, HIV-1 pre-exposure significantly increased HCV transmission by LCs. HIV-1 replication is crucial for the increased HCV transmission as treating *ex vivo* tissue with HIV-1 replication-inhibitors significantly decreased HIV-1-induced HCV transmission. Next we identified the mechanism in HIV-1 exposed LCs. Notably, HCV transmission by HIV-1 exposed LCs was dependent on Syndecan-4, as silencing of Syndecan-4 abrogated transmission. The role of Syndecan-4 in HCV transmission was further confirmed using cell-lines expressing Syndecan-4. Furthermore, we showed that activation of LCs enhanced Syndcan-4-mediated transmission of HCV, which supports studies showing that other sexually acquired infections, increase HCV susceptibility. Thus, our data strongly suggest that HIV-1 replication as well as immune activation in mucosal tissues in HIV-1 infected MSM, changes LC function, allowing Syndecan-4 to capture and subsequently transmit HCV to hepatocytes. This novel transmission mechanism implicates also that the activation state of LCs is an important determinant for HCV susceptibility after sexual contact.This work was supported by Aidsfonds, grant number: 2014014

WS.D4.04.05

DUSP4 regulates STING and RIG-I mediated signalling in response to virus infection

H. Jiao, S. J. James, ***Y. Zhang****;*
National University of Singapore, Singapore, Singapore.

Detection of cytosolic nucleic acids by pattern recognition receptors including STING and RIG-I leads to activation of multiple signalling pathways that culminate in the production of type I interferons (IFNs) which are vital for host survival during virus infection. In addition, type I IFNs are also associated with autoimmune diseases. Hence, it is important to elucidate the mechanisms that govern their expression. In this study, the critical regulatory function of DUSP4 in innate immune signalling was demonstrated. It was found that DUSP4 knockout (KO) macrophages expressed increased level of type I IFNs in response to RIG-I and STNG activation, as well as influenza and HSV-1 infection compared to wildtype (WT) cells. This increased type I IFN expression in KO cells was associated increased activation of ERK and TBK1-IRF3 in KO cells compared to WT cells. Mice deficient in DUSP4 are more resistant to both RNA and DNA virus infection but are more susceptible to malaria compared to control. Mechanistically, DUSP4 inhibits TBK1 and ERK2 activation to suppress type I IFN production. Therefore, our study not only established DUSP4 as a common regulator of nucleic acid sensor signalling, but also shed light on the regulation of the type I IFN system.

WS.D4.04.06

A lncRNA promotes viral replication by modulating host cellular metabolism

P. Wang[1], *J. Xu*[1], *Y. Wang*[1], *X. Cao*[1,2,3];
[1]*Institute of Immunology, Second Military Medical University, Shanghai, China,* [2]*Institute of Immunology, Zhejiang University School of Medicine, Hangzhou, China,* [3]*Department of Immunology & Center for Immunotherapy, Institute of Basic Medical Sciences, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China.*

Viruses have evolved to alter the host metabolic pathways to ensure optimal niche for viral replication and survival. Identification of how viruses alter the host cellular metabolism is necessary for understanding viral infection and revealing targets for antiviral therapeutics. We previously demonstrated that long-non-coding RNAs (lncRNAs) regulate dendritic cell differentiation, and therefore, have a pleiotropic effect to the host immune system (1). Here we hypothesized that viruses utilize lncRNA to alter the host cellular metabolism that provides necessary energy and material for viral replication.
In this study, we identified that a group of lncRNAs were induced by the infections from a number of viruses. Functional screening demonstrated that the expression of one of these viral-induced lncRNAs, lncRNA-ACOD1, enhanced viral replication in both murine and human cells. In lncRNA-ACOD1 knockout mice, viral infection was significantly reduced in an IFN-independent manner. Gene expression profiling and metabolomics surveys revealed that viral infections led to significant host metabolic changes in wildtype hosts but the lncRNA-ACOD1 knockout totally abolished the metabolic alteration. Mechanistically, we demonstrated that lncRNA-ACOD1 directly binds to a metabolic enzyme, GOT2. The binding region on GOT2 is spatially adjacent to its substrate-binding site, and thus, the binding of lncRNA-ACOD1 enhanced GOT2's catalytic activity. The treatment of GOT2 or its metabolites restored viral replication in lncRNA-ACOD1 knockout mice, demonstrating that lncRNA-ACOD1-mediated viral replication is dependent on GOT2 catalysis. This work revealed a novel feedback mechanism of viral infection by altering host cellular metabolism through a lncRNA.
(1) Wang et al., Science 344, 310(2014):310-313.

WS.D4.05 Innate-adaptive interface during microbial infections

WS.D4.05.01

Targeting newly identified pathogenic monocyte subsets with immune-modulatory treatment resolves severe malaria

P. Niewold[1], *A. Cohen*[1], *C. van Vreden*[2], *D. R. Getts*[3], *G. Grau*[1], *N. King*[1,2];
[1]*University of Sydney, Sydney, Australia,* [2]*Ramaciotti Facilty for Human System Biology, Sydney, Australia,* [3]*Northwestern University, Chicago, United States.*

Severe malaria is caused by the mosquito-borne parasite *Plasmodium falciparum* and is associated with lethal complications including acute respiratory distress syndrome and cerebral malaria (CM). Current treatment has limited efficacy in advanced infection as it is primarily directed at the parasite. This fails to address the neurological damage and respiratory distress caused by host blood cell accumulation in the brain microvasculature and lung interstitium. Therefore, we sought to identify cells contributing to pathology as novel targets for immunemodulatory therapies.
In a preclinical mouse model of CM, highdimensional flow cytometry and computational analysis identified Ly6C^{hi}monocytederived Ly6C^{lo}monocytes and interstitial macrophages as the main pathological populations in the brain and lungs, respectively. Previous studies have shown immune modifying particles (IMP) target Ly6C^{hi}monocytes, preventing their migration to inflammatory foci. To this end, we treated mice with neurological signs of CM with 2 doses of IMP in combination with WHOrecommended anti-parasitic artesunate. This combination therapy resulted in 88% survival in animals that would otherwise succumb to disease within 36 hours. Treatment effectively reduced the accumulation of cells in the brain vasculature and lung, resulting in clearance of parasitaemia and immunity.
In conclusion, through detailed analysis of the immune response in severe malaria, we identified principal pathogenic immune subsets and targeted them with a novel combination therapy, resulting in increased survival in an otherwise lethal syndrome. This is the first specific immunemodulatory treatment addressing both hostmediated pathology and the parasite in severe malaria, revealing a novel avenue for human treatment.

WS.D4.05.02

The use of mass cytometry to study type 2 immune responses in the context of helminth infections

K. de Ruiter[1], *D. L. Tahapary*[1,2], *K. Stam*[1], *V. van Unen*[1], *E. Sartono*[1], *J. W. Smit*[3,1], *T. Supali*[2], *M. Yazdankbakhsh*[1];
[1]*Leiden University Medical Center, Leiden, Netherlands,* [2]*Universitas Indonesia, Jakarta, Indonesia,* [3]*Radboud University Medical Center, Nijmegen, Netherlands.*

Studies have shown that helminths can have a profound role in shaping immune responses to vaccines, allergens or autoantigens by inducing strong type 2 and regulatory responses. We used mass cytometry to gain insight into the immunomodulatory effects of helminths, by performing unbiased immune profiling of Indonesian adults who were infected with soil-transmitted helminths, before and 1 year after anthelmintic treatment, as well as healthy European volunteers. The use of our recently developed Cytosplore to analyse high-dimensional single-cell immunological data, allowed the identification of very distinct immune signatures in Europeans and Indonesians. Higher frequencies of Th2 cells (in particular CD161^{+} Th2 cells), ILC2s and regulatory T cells expressing CTLA-4 and ICOS were found in Indonesians that were infected, compared to Europeans. Except for ILC2s, the frequencies of these cell subsets decreased after 1 year of anthelmintic treatment. In addition, we assessed the functional capacity of cells, and observed a clearly higher production of Th2 cytokines upon stimulation with PMA/Ionomycin in infected Indonesians compared to Europeans, which was decreased after deworming. Subsets of CD4^{+} and CD8^{+} T cells, γδ T cells and ILC2s showed to be the main producers of Th2 cytokines. These results provide us with a detailed insight into the types of cells that participate in the strong type 2 and regulatory response induced by helminths.
This study was funded by the Royal Netherlands Academy of Arts and Science (KNAW).

WS.D4.05.03

The Notch signaling pathway controls basophil responses during helminth-induced type 2 inflammation

***L. M. Webb**, S. A. Peng, O. O. Oyesola, R. L. Cubitt, J. K. Grenier, C. G. Danko, E. D. Tait Wojno;*
Cornell University, Ithaca, United States.

Type 2 inflammation is characterized by production of the cytokines IL-4, IL-5 and IL-13 and promotes clearance of gastrointestinal helminth parasites, which infect over 2 billion people worldwide. Basophils are innate immune cells that drive expulsion of the murine helminth *Trichuris muris*. However, the molecular mechanisms that control basophil function and gene expression during helminth-induced type 2 inflammation remain unclear. We show that during *T. muris* infection, basophils localized to the intestine and upregulated components of the Notch signaling pathway, which regulates gene expression programs during development and inflammation. *In vitro*, Notch inhibition abrogated IL-33-elicited basophil IL-4 and IL-6 production by directly targeting *Il4* and *Il6*. Transcriptional profiling of Notch-deficient basophils revealed that Notch directs basophil responsiveness to inflammatory cues and effector gene expression. *In vivo*, Notch-deficient basophils did not localize effectively within the lamina propria of the intestine, at the site of infection, and displayed decreased interaction with $CD4^+$ cells. Consequently, mice lacking basophil-intrinsic Notch signaling had impaired worm clearance and decreased intestinal type 2 inflammation following *T. muris* infection. These findings demonstrate that Notch regulates basophil gene expression and effector function during helminth-induced type 2 inflammation, with repercussions for our understanding of type 2 immunity and for development of effective therapeutics aimed at this arm of host defense.

WS.D4.05.04

Type I Interferons Suppresses Anti-parasitic $CD4^+$T Cell Responses in Visceral Leishmaniasis

***R. Kumar**[1], P. Bunn[2], F. Rivera[2], N. Singh[3], S. Sundar[3], C. Engwerda[2];*
[1]Institute of Science, Banaras Hindu University, Varanasi, India, [2]QIMR Berghofer Medical Research Institute, Brisbane, Australia, [3]Institute of Medical Science, Banaras Hindu University, Varanasi, India.

Introduction: Many pathogens, including viruses, bacteria, and protozoan parasites, suppress cell mediated immune responses through activation of type I Interferon (IFN-1) signalling. However, the role of IFN-1 during *Leishmania donovani* infection causing visceral leishmaniasis (VL) is not well known. Here we report that IFN-1 plays an important role in the pathogenesis of VL by impairing parasite clearance and suppressing pro-inflammatory cytokine production. **Methods:** Mice lacking type-1 IFN signalling (B6.IFNαR1$^{-/-}$ mice) and wild type (WT) C57BL/6 mice were infected intra-venously with $2x10^7$ *L. donovani* amastigotes. Parasite burden was measured at day 14, 28 and 56 post infections and serum cytokine levels was analysed. Intracellular cytokine staining and flow cytometry was performed to detect the $CD4^+$ T cell-derived IFN-γ production. Peripheral blood mononuclear cells (PBMCs) from VL controls were also collected to measure mRNA encoding IFN-1 related genes and whole blood assay was employed to measure the antigen specific immune response after IFN-1 signalling blockade. **Results:** B6.IFNαR1$^{-/-}$ mice showed enhanced pro-inflammatory cytokine production and better control of parasite burden in liver and spleen. IFN-1 signalling suppressed $CD4^+$ T cell-derived IFN-γ production and prevented Th1 response from controlling parasite replication. Studies in VL patients supported these findings and showed enhanced accumulation of mRNA encoding type I IFN signature genes in PBMCs that were reduced following successful drug therapy. The blockade of type-1 IFN signalling enhanced antigen specific IFN-γ production. **Conclusion:** Together, these results identify type-1 IFN signalling pathways as a potential therapeutic target to treat VL by enhancing anti-parasitic $CD4^+$ T cell responses.

WS.D4.05.05

Cross-presentation by pDC of cell-associated HIV is potentiated by non-cognate $CD8\ T^+$ cell pre-activation

***E. X. Hatton**, S. Isnard, M. Iannetta, J. Guillerme, A. Hosmalin;*
Institut Cochin, INSERM U1016, CNRS UMR8104, Université Paris Descartes, Sorbonne Paris Cité, Paris, France.

Human Plasmacytoid DC (pDC) cross-present antigens from apoptotic HIV infected cells, which contain both antigen and microbial stimuli. We studied MHC-restricted cognateand innate mechanisms leading to this cross-presentation.
We used HLA-A2 restricted HIV-Gag_{77-85}-specific $CD8^+$ T cell clones, UV-C-irradiated HIV-infected or uninfected H9 cells as antigen donor cells, and saquinavir to block direct viral presentation. DC populations were immunomagnetically and FACS-sorted to >95% purity, from HLA-A2+ or - donor buffy coats. We tested IFN-γ secretion by ELISA or intracellular production by flow cytometry.
IFN-γ secretion in response to cross-presentation of apoptotic H9-HIV cells by pDC was fully MHC-class I restricted, but intracellular IFN-γ production only partly. Inhibitors of HIV TLR-7 signaling strongly decreased the innate, non MHC-restricted intracellular IFN-γ production. PDC cultured with H9-HIV cells produced different cytokines. Among those, recombinant IFN-α or β, but not TNF-α, added to the $CD8^+$ T cell clones, in the absence of H9-HIV cells or pDC, induced intracellular IFN-γ production; they enhanced IFN-γ secretion triggered by cell-free, cognate stimulation through CD3 and CD28-bound beads. Conversely, neutralizing antibodies against these cytokines abolished the non-MHC-restricted intracellular IFN-γ production. Compared to pDC, purified cDC1 or cDC2 required additional TLR-3 or -2/4 stimulation to cross-present antigens from H9-HIV cells. Indeed, H9-HIV apoptotic cells induced maturation of pDC, but not cDC.
Our results show that pDC can detect HIV-infected cells by producing type I IFNs, which pre-activate $CD8^+$ T cell IFN-γ production and increase its release upon cognate interaction.
Support: ANRS, ANR-10-LABX-62-IBEID, INSERM, CNRS, Univ Paris Descartes, SIMIT

WS.D4.05.06

Memory CD8 T cell inflation versus tissue resident memory T cells: same patrollers, same controllers?

***S. P. M. Welten**, A. Oxenius;*
Institute of Microbiology, ETH Zürich, Zürich, Switzerland.

The induction of memory CD8 T cells residing in peripheral tissues is of considerable interest for T cell based vaccines as they can immediately exert effector functions and thus provide protection in case of pathogen encounter at mucosal and barrier sites. Cytomegalovirus (CMV)-based vaccines support the induction and accumulation of large amounts of effector memory CD8 T cells in peripheral tissues, a process called memory inflation. Tissue resident memory (T_{RM}) T cells are another subset of cells that take long-term residence in peripheral tissues. Both populations have gained substantial interest in exploiting for vaccine purposes; however, it is unclear which population is superior in providing long-term protection against a variety of pathogens and tumors. Here we compared the localization, maintenance and protective capacity of these distinct T cell subsets in the lungs. We found that inflationary T cells and T_{RM} cells have a unique phenotype and occupy distinct niches in the lung tissue. However, upon antigen re-challenge, inflationary T cells were able to adapt their phenotype and location. While the inflationary T cell pool was stably maintained for a prolonged period and dependent on IL-15-mediated signals, the number of T_{RM} cells in the lungs gradually declined over time. The different characteristics of T_{RM} and inflationary T cells also translated in distinct capacities to control various viral infections. Together these results bear relevance for vaccines aimed at eliciting memory T cells at mucosal sites.

WS.D4.06 Bacterial infections and immune activation

WS.D4.06.01

A novel mechanism of neutrophil entry into epithelial cells is involved in damping inflammation but exposes a backdoor for bacterial invasion

***N. Y. SHPIGEL**;*
Hebrew University, Rehovot, Israel.

Neutrophil mobilization is a crucial response to protect the host against invading microorganisms. Neutrophil recruitment and removal is tightly regulated through apoptosis and phagocytosis by resident and recruited macrophages. Nevertheless, in many organs, macrophage mobilization across the barrier epithelium is dearth and cannot be a major mechanism for homeostasis of inflammation. We suggest that in some mucosal surfaces or barrier epithelium, like urinary and gall bladder or the mammary alveoli, phagocytosis of apoptotic neutrophils by macrophage may not be the only or most important mechanism of neutrophil safe disposal and homeostasis of inflammation. Here we propose a novel and previously unrecognized mechanism of neutrophil internalization and apoptotic death program in epithelial cells. Viable neutrophils trigger a mechanism that enables them to crawl into cytoplasmic double membrane compartments in the host cells. Next, internalized neutrophils lose the membrane compartment and undergo apoptotic cell death in the cytoplasm. We were able to demonstrate this incredible phenomena both in vitro on epithelial cell lines and in murine in vivo systems. This mechanism contributes to safe disposal of neutrophils and for the resolution of inflammation in some organs and disease processes. Moreover, some pathogenic bacteria take advantage of neutrophil cell invasion process, using it to invade epithelial cells, where they can proliferate to form intracellular bacterial communities. Bacteria invasion to epithelia may be of prime importance in the pathogenesis of major diseases such as bovine mastitis, urinary tract infection and typhoid fever, and might account for the chronic carriage and relapsing disease.

WS.D4.06.02

Lung surfactant lipids provide immune protection against non-typeable *Haemophilus influenzae* respiratory infection

B. Garcia-Fojeda[1,2], *Z. Gonzalez-Carnicero*[2], *A. De Lorenzo*[2], *C. Minutti*[1,2,3], *B. Euba*[1,4], *A. Iglesias-Ceacero*[2], *S. Castillo-Lluva*[2], *J. Garmendia*[1,4], *C. Casals*[1,2];
[1]*CIBER de Enfermedades Respiratorias, Madrid, Spain,* [2]*Universidad Complutense de Madrid, Madrid, Spain,* [3]*The Francis Crick Institute, London, United Kingdom,* [4]*Instituto de Agrobiotecnología, CSIC-Universidad Pública de Navarra, Mutilva, Spain.*

Non-typeable *Haemophilus influenzae* (NTHi) causes persistent respiratory infections in immunocompromised patients likely linked to its capacity to invade and survive within pneumocytes. In the alveolar fluid, NTHi is in contact with pulmonary surfactant, a lipoprotein complex that prevents alveolar collapse and constitutes the front line of defense against inhaled pathogens. The objective of this study was to investigate the effect of surfactant phospholipids on the host-pathogen interaction between NTHi and pneumocytes. For this purpose, we used two types of surfactant lipid vesicles present in the alveolar fluid: i) multilamellar vesicles (MLVs, > 1 μm diameter), which constitute the tensioactive material of surfactant, and ii) small unilamellar vesicles (SUVs, 40 nm diameter), which are generated after inspiration/expiration cycles, and are endocytosed by pneumocytes for their degradation and/or recycling. Results indicated that MLVs of surfactant inhibited adhesion of NTHi to pneumocytes and, consequently, NTHi invasion. In contrast, endocytosed surfactant lipids did not affect NTHi adhesion but inhibited entry of NTHi in pneumocytes. SUVs of lung surfactant were endocytosed via the scavenger receptor SR-BI, but not CD36, and anti-SR-BI antibodies abrogated surfactant inhibition of NTHi invasion. Endocytosis of SUVs inhibited Akt phosphorylation and Rac1 GTPase activation, key events in NTHi internalization. Lung surfactant administration in a mouse model of NTHi pulmonary infection amplified bacterial clearance, supporting the protective role of surfactant lipids against NTHi infection. These results suggest that decreased surfactant lipid levels reported in smokers and patients with chronic obstructive pulmonary disease may increase their susceptibility to infection by NTHi. Funding: SAF-2015-65307-R.

WS.D4.06.03

CD200R1L, a novel activating receptor on human neutrophils that induces ROS and NET release

M. Ramos[1], *J. Stok*[1], *C. Kesmir*[2], *T. Jansen*[1], *L. Meyaard*[1], *M. van der Vlist*[1];
[1]*UMC Utrecht; Oncode Institute, Utrecht, Netherlands,* [2]*Utrecht University, Utrecht, Netherlands.*

Inhibitory and activating receptors play a key role in modulating the amplitude and duration of immune responses during infection and are central in achieving immune balance in homeostatic conditions. One example is the paired CD200R gene family, which in humans comprises of one inhibitory, CD200R, and one putative activating member, CD200R1L. In contrast to literature, where has been reported that the activating CD200R1L is not expressed as a protein, we demonstrated that CD200R1L is endogenously expressed by human neutrophils. Our functional data show that this activating receptor can induce important cellular functions such as formation of reactive oxygen species (ROS) and release of extracellular traps (NETosis). Cross-linking CD200R1L with a monoclonal antibody elicited an FcγR-independent oxidative burst on primary neutrophils that relied on Syk, PI3Kβ, PI3Kδ and Rac GTPase signalling. By making use of the ROS inhibitor DPI we determined that CD200R1L elicited a classical NETosis process dependent on ROS generation by NADPH oxidase. Phylogenetic studies showed that CD200R1L was present in all examined species, implying that evolutionary conservation of this receptor was critical for protection against co-evolving pathogens. With few exceptions, activating CD200R1L receptors had longer branch lengths than inhibitory ones suggesting that activating receptors accumulated mutations at a faster pace. The faster evolution of these receptors agrees with the proposed theory that under pathogen pressure, activating receptors evolve quicker which would fit this receptor's functional capabilities. Based on our data, we hypothesize that CD200R1L represents a hitherto unidentified pathogen-recognition receptor on human neutrophils important for pathogen clearance.

WS.D4.06.04

Innate immune recognition of *Staphylococcus aureus* by dendritic cells is affected by antibiotic resistance mutations

T. P. Patton[1,2], *J. Jiang*[2,3], *R. J. Lundie*[1,2], *V. Oorschot*[2,4], *G. Ramm*[2,4], *A. Y. Peleg*[2,3,5], *M. A. O'Keeffe*[1,2];
[1]*Infection and Immunity Program, Monash Biomedicine Discovery Institute, Monash University, Melbourne, Australia,* [2]*Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Australia,* [3]*Department of Microbiology, Monash University, Melbourne, Australia,* [4]*Monash Ramaciotti Centre for Cryo Electron Microscopy, Monash University, Melbourne, Australia,* [5]*Department of Infectious Diseases, The Alfred Hospital and Central Clinical School, Monash University, Melbourne, Australia.*

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) represents an emerging public health threat. Resistance to last-line antibiotics is on the rise, correlating with increased morbidity and mortality in the hospital setting. Recently, whole genome sequencing revealed that mutations conferring resistance to last-line antibiotics affect the composition of the bacterial cell membrane and wall. Here we investigate how these mutations affect innate immune recognition by dendritic cells (DCs) of the host.
Methods: Paired isogenic clinical isolates were obtained from patients suffering chronic infection with MRSA. Primary DC subsets were phenotyped in response to these bacterial stimuli; characterising inflammatory cytokine secretion, as well as surface phenotype. Differences in antigen uptake and trafficking by DC subsets were quantified via a combination of flow cytometry, electron microscopy and live cell imaging techniques.
Results: The acquisition of daptomycin resistance is associated with a decrease in immunological recognition by dendritic cells (DCs). This reduction reflects decreased expression of co-stimulatory markers CD40 and CD80, alongside a significant decrease in production of pro-inflammatory cytokines and chemokines. Furthermore, the recreation of individual point mutations in genes responsible for phospholipid biosynthesis found in resistant MRSA strains play a critical role in modulating immune recognition of resistant strains by DCs.
Conclusion: These findings highlight of a novel aspect of antibiotic resistance- showing for the first time that point mutations arising in MRSA during antibiotic therapy can result in decreased immunological recognition by dendritic cells.
This work is supported by the Cass Foundation and the Australian Research Training Program.

WS.D4.06.05

Hemolysin liberates bacterial outer membrane vesicles for cytosolic lipopolysaccharide sensing

S. Chen[1], *Q. Liu*[1,2,3];
[1]*State Key Laboratory of Bioreactor Engineering, ShangHai, China,* [2]*Qingdao National Laboratory for Marine Science and Technology, Qingdao, China,* [3]*Shanghai Engineering Research Center of Maricultured Animal Vaccines, Shanghai, China.*

Induction: Inflammatory caspase-11/4/5 recognize cytosolic LPS from invading Gram-negative bacteria. Since extracellular or vacuole-constrained bacteria are thought to rarely access the cytoplasm, how their LPS are exposed to the cytosolic sensors is a critical event for pathogen recognition. Hemolysin was generally accepted to rupture cell membrane. Whether and how hemolysin participates in non-canonical inflammasome signaling remains uncovered.
Materials and Methods: LDH and cytokine release induced by bacterial or OMV infection were measured by a cytotoxicity or an ELISA kit, respectively. Subcellular cell fractions were extracted by a digitonin-based fractionation for LPS quantification. For OMVs-containing vacuole integrity detection, we constructed an eukaryotic expression plasmid for GFP tagged galectin-3 introduced into HeLa cells to observe the specks of galectin recruitment. In vivo, an oral infection model was used to detect bacterial colonization, gut inflammation, and enterocyte pyroptosis.
Results: Hemolysin-overexpressed enterobacteria triggered significantly increased caspase-4 activation in human intestinal epithelial cells. Hemolysin promoted LPS cytosolic delivery from extracellular bacteria through dynamin-dependent endocytosis. Further, hemolysin was largely associated with bacterial outer membrane vesicles (OMVs) and induced rupture of OMV-containing vacuoles, subsequently increasing LPS exposure to the cytosolic sensor. Accordingly, overexpression of hemolysin promoted caspase-11 dependent IL-18 secretion, gut inflammation, and enterocyte pyroptosis in orally-infected mice, which was associated with restricting bacterial colonization in vivo.
Conclusions: Hemolysin promotes noncanonical inflammasome activation via liberating OMVs for cytosolic LPS sensing, which offers insights into innate immune surveillance of dysregulated hemolysin via caspase-11/4 in intestinal antibacterial defenses.
(National Natural Science Foundation of China, No. 31622059)

WS.D4.06.06

Analysis of CD6-based peptides in experimental sepsis

C. Català[1], *M. Martínez-Florensa*[1], *M. Velasco-de Andrés*[1], *O. Cañadas*[2,3], *V. Fraile-Ágreda*[2,3], *S. Casadó-Llombart*[1], *N. Armiger-Borràs*[1], *M. Consuegra-Fernández*[1], *C. Casals*[2,3], *F. LOZANO*[1,4,5];

[1]*Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), BARCELONA, Spain,* [2]*Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, MADRID, Spain,* [3]*Departmento de Bioquímica y Biología Molecular, Universidad Complutense de Madrid, Madrid, Spain,* [4]*Servei d'Immunologia, Centre de Diagnòstic Biomèdic, Hospital Clínic de Barcelona, Barcelona, Spain,* [5]*Departament de Biomedicina, Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain.*

Sepsis still constitutes one of the most important causes of death worldwide, a fact aggravated by the appearance of multidrug resistant strains due to indiscriminate use of antibiotics. Receptors from the immune innate system recognize pathogen-associated molecular patterns (PAMPs) and can be a source of antimicrobial therapies alternative/adjunctive to antibiotics. CD6 is a lymphocyte-specific surface receptor of the scavenger receptor cysteine-rich superfamily (SRCR-SF) displaying bacterial-binding properties through recognition of PAMPs from Gram positive (lipoteichoic acid; LTA) and negative (lipopolysaccharide; LPS). The present work analysed the bacterial-binding properties of three conserved short peptides (11-mer) mapping at surface accessible locations from each of the three extracellular SRCR domains of human CD6 (CD6.PD1, GTVEVRLEASW; CD6.PD2 GRVEMLEHGEW; and CD6.PD3, GQVEVHFRGVW). All peptides showed relative high binding affinities for both LPS (K_d from 3.5 to 3,000 nM) and LTA (K_d from 36 to 680 nM). CD6.PD1 and CD6.PD3 peptides, but not CD6.PD2, also showed broad *in vitro* bacterial-agglutination properties.
In *in vivo* studies, the CD6.PD3 peptide excelled by dose- and time-dependent improving the survival of mice undergoing cecal ligation and puncture (CLP)-induced sepsis, a fact concomitant with decreased pro-inflammatory cytokine serum levels and bacterial load. CD6.PD3 also showed additive effects on survival of CLP-septic mice when combined with the broad spectrum bactericidal antibiotic Imipenem/Cilastatin. These results illustrate the therapeutic potential of peptides retaining the bacterial-binding properties of native CD6. Supported by Spanish MINECO (SAF2016-80535- R; PCIN-2015-070) and ISCIII [RD12/0015/0018 and CIBERES CB06/06/0002] -co-financed by European Development Regional Fund "A way to achieve Europe".

WS.D4.07 Innate immune responses and infection

WS.D4.07.01

Primary neutrophil defects reveal the mechanisms of neutrophil extracellular trap (NET) formation and NETosis: a unique role for active cytoskeletal rearrangments

E. Sprenkeler[1], *A. Tool*[1], *T. K. van den Berg*[1], ***T. Kuijpers***[1,2];

[1]*Department of Blood Cell Research, Sanquin Research, and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands,* [2]*Emma Children's Hospital, Amsterdam University Medical Center (AUMC), University of Amsterdam, Amsterdam, Netherlands.*

Although the formation and microscopic appearance of neutrophil extracellular traps (NETs), the physiological mechanisms triggering NET release and the concomitant cell death known as NETosis, have been studied since has been studied since the first report in 2004. The relevance of NET formation to disease and clinical translatability of this unconventional cellular mechanism remain poorly understood. Using neutrophils of patients with various rare genetically well-characterized disorders as human knockout models (including Chronic Granulomatous disease [CGD], granule deficiencies [GPS], degranulation defects [FHL5], ARPC1B and MKL1 deficiency), we have been able to unravel the consecutive steps required for NETosis to occur. Additional use of small compounds to inhibit specific cellular signaling pathways in neutrophils from healthy controls were used to verify the role of these mechanisms identified in patient neutrophils. Time courses indicated differences in the moment certain processes became active from cellular activation until DNA release using microcopy, Sytox Green kinetic determination and live cell imaging during the process of NETosis.
In this way, we have been able to dissect two phases and the signaling steps involved in the overall process of NETosis. In either of these two phases, our studies demonstrate that reactive oxygen species, serine proteases as well as specific granule components and the rearrangements of cytoskeletal elements have an essential and unique contribution in the active DNA release from human neutrophils. These data will be discussed in the view of the clinical manifestations.

WS.D4.07.02

Characterization of neutrophils generated in vitro from Hoxb8-transduced myeloid progenitor cells

A. Orosz, *A. Mócsai;*
Semmelweis University, Budapest, Hungary.

Background: Acute inflammation and neutrophil granulocytes have long been mentioned together, however the molecular background of the neuthrophils' function is still mostly unknown. Therefore we are hoping to uncover these molecular mechanisms using ex vivo generated neutrophil cells from the so called SCF ER-Hoxb8 progenitors. These bone marrow-derived progenitors are retrovirally transduced to express the Hoxb8 transcription factor in the presence of β-estrogen, in order to keep them in progenitor state for long periods of time. From them, unlimited amounts of neutrophils can be differentiated using certain cytokines. **Materials and methods**: Neutrophils were generated via growing progenitors in β-estrogen free medium supplemented with G-CSF. Cell surface markers were detected using flow cytometry. ROS production was measured according to cytochrome-c reduction. Adhesion and migration capabilities were tested using different stimulating agents. Fagocyting properties were measured using fluorescent *Staphylococcus aureus* and *Candida albicans*. **Results**: $CD45^+$ progenitor cell line can be cultured for long time.
$Ly6G^+$ neutrophils start to differentiate in 4 days. 5-6 days old neutrophils show strong adhesion upon PMA and IC stimulation. Hoxb8 neutrophils can also produce ROS upon various stimuli in vitro. Neutrophils appear in the circulation after adoptive transfer of progenitors into lethally irradiated recipient mice. In vivo generated neutrophils can migrate and carry out phagocytosis in inflammatory conditions. **Conclusions**: The SCF ER-Hoxb8 cell line can be a great alternative to the common- genetically modified mice-based methods in use to discover the molecular basis of the role of the neutrophils in inflammatory diseases both in vitro and in vivo.

WS.D4.07.03

Regnase-3 is an essential RNase for the Interferon pathway in tissue macrophages

M. Von Gamm[1], *A. Schaub*[1], *J. Lichti*[1], *M. Tschöp*[1], *V. Hornung*[2], *C. Schulz*[3], *M. Heikenwälder*[4], ***E. Glasmacher***[1];
[1]*Helmholtz Center Munich, Munich, Germany,* [2]*Gene Center, Munich, Germany,* [3]*LMU, Munich, Germany,* [4]*DKFZ, Heidelberg, Germany.*

Regnases are immune cell-expressed RNases. Regnase-1 degrades cellular and viral RNAs in macrophages and T cells, but the role of Regnase-3 remains unknown. Regnase-3-deficient (*Reg3*$^{-/-}$) mice develop severe lymphadenopathy, caused by extramedullary hematopoiesis and increased type-I-interferon signaling. CD19, as well as CD4- specific ablation of Regnase-3 in mice does not result in phenotypic changes, however LysM-specific deletion recapitulates this phenotype. Regnase-3 protein is regulated via IKK upon TLR activation, similarly to Regnase-1. However, Regnase-3 steady-state expression is specifically high in macrophages and in non-lymphoid tissues, different to Regnase-1. Whereas Regnase-1 transcription is controlled via NF-kB activity, Regnase-3 is transcriptionally regulated via TLR3 and TBK signaling. Although Regnase-3 deficiency does not affect phagocytic activity, it localizes to phagosomes in macrophages, is regulated via lysosomal degradation upon RNA agonist stimulation and interacts with TLR3. Therefore, Regnase-3 is the evolutionary counterpart to Regnase-1, functioning in the endocytosis and interferon pathway, ensuring tissue homeostasis.

WS.D4.07.04

A viral immunoevasin controls innate immunity by targeting the prototypical natural killer cell receptor family

O. Aguliar[1], ***R. Berry***[2], *M. Rahim*[3], *J. Reichel*[4], *B. Popovic*[4], *M. Tanaka*[1], *Z. Fu*[2], *G. Balaji*[2], *T. Lau*[1], *M. Tu*[3], *C. Kirkham*[1], *A. Mahmoud*[3], *A. Mesci*[1], *A. Krmpotic*[4], *D. Allan*[1], *A. Makrigiannis*[3], *S. Jonjic*[4], *J. Rossjohn*[2], *J. Carlyle*[1];
[1]*University of Toronto, Toronto, Canada,* [2]*Monash University, Clayton, Australia,* [3]*Univeristy of Ottawa, Ottawa, Canada,* [4]*University of Rijeka, Rijeka, Croatia.*

Natural killer (NK) cells play a key role in innate immunity by detecting alterations in self and non-self ligands via paired NK cell receptors (NKRs). Despite identification of numerous NKR-ligand interactions, physiological ligands for the prototypical NK1.1 orphan receptor remain elusive. Here, we identify a viral ligand for the inhibitory and activating NKR- P1 (NK1.1) receptors. This murine cytomegalovirus (MCMV)-encoded protein, m12, restrains NK cell effector function by directly engaging the inhibitory NKR-P1B receptor. However, m12 also interacts with the activating NKR-P1A/C receptors to counter-balance m12 decoy function. Structural analyses reveal that m12 sequesters a large NKR-P1 surface area via a ''polar claw'' mechanism. Polymorphisms in, and ablation of, the viral m12 protein and host NKR-P1B/C alleles impact NK cell responses in vivo. Thus, we identify the long-sought foreign ligand for this key immunoregulatory NKR family and reveal how it controls the evolutionary balance of immune recognition during host-pathogen interplay.

WS.D4.07.05

Intracellular lifestyle of the probiotic bacteria *Lactobacillus plantarum*; implications for its extraintestinal dissemination

A. Pellon[1], *E. Atondo*[1], *M. Montesinos-Polledo*[1], *M. A. Pascual-Itoiz*[1], *A. Peña-Cearra*[1,2], *A. Carreras-Gonzalez*[1], *S. Varona-Fernandez*[3], *J. M. Landete*[4], *J. M. Rodriguez*[5], *R. Prados-Rosales*[1,6], *L. Abecia*[1], *J. L. Lavin*[3], *J. Anguita*[1,7], *H. Rodriguez*[1];
[1]*Macrophage and Tick Vaccine Laboratory, CIC bioGUNE, Derio, Spain,* [2]*Faculty of Science and Technlogy, University of the Basque Country (UPV/EHU), Leioa, Spain,* [3]*CIC bioGUNE, Derio, Spain,* [4]*Departamento de Tecnología de Alimentos, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain,* [5]*Department of Nutrition, Food Science and Food Technology, Complutense University of Madrid, Madrid, Spain,* [6]*Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, United States,* [7]*Ikerbasque, Basque Foundation for Science, Bilbao, Spain.*

The interaction of the immune system and microbiota is paramount for the development of the immune system and the maintenance of gut homeostasis. In recent years bacterial symbionts have been found in previously considered sterile body sites, including human breastmilk. Therefore, it seems clear that translocation of bacteria occurs physiologically and this has been hypothesized to be controlled by immune cells. Here, we explore the ability of the probiotic bacterium *Lactobacillus plantarum* to survive within macrophages and provide some of the mechanisms involved in this process.
We show the ability of *L. plantarum* strains to survive within the RAW264.7 macrophage-like cell line up to 24 h and, to a lesser extent, inside mouse bone marrow-derived macrophages and human monocyte-derived macrophages. In the absence of antibiotics, *L. plantarum* strains are capable of extruding from immune cells after internalization, which is consistent with the extraintestinal translocation hypothesis. We also addressed the contribution of the complement system in *L. plantarum* internalization and survival: we found that both processes are dependent on bacterial opsonization and recognition by macrophage complement receptor 3. Additionally, by using human whole blood as a more complex model we observed that monocytes are capable of internalizing and sustaining a significant *L. plantarum* population.
Collectively, our work provides one of the first evidences of the capacity of probiotic bacteria to survive within macrophages, which we proved to be complement system-dependent. Therefore, we propose that monocytes/macrophages are important players during physiological bacterial translocation.

WS.D4.07.06

Nanovaccines to prevent listeriosis in the elderly

C. Alvarez-Dominguez[1], *R. Calderon-Gonzalez*[1], *H. Teran-Navarro*[1], *D. Salcines-Cuevas*[1], *M. Fresno-Escudero*[2];
[1]*Instituto de Formacion e Investigación Marques de Valdecilla, Santander, Spain,* [2]*Centro de Biología Molecular Severo Ochoa, Madrid, Spain.*

Introduction: Clinical cases of listeriosis in the elderly associate with meningitis or septicaemia. Listeriosis severe cases have increased in European countries, Spain in particular urging for safe vaccines. We have prepared new nanovaccine formulations for *Listeria monocytogenes* (LM) including two different adjuvants. Methods: We have *i.v* vaccinated old-mice, young adults and pregnant mothers and challenged them with a sub-lethal dose of wild type LM. Brains, livers in neonates and livers and spleens in adult mice are examine for viable bacteria, immune responses and cytokine profiles. We also collected clinical listeriosis cases and explore bacterial virulence and immune responses in sera and monocyte derived dendritic cells (MoDC). Results: Old-mice and young adults become protected with nanovaccine formulations using adjuvants that expands CD4 and CD8 immune responses, while neonates were better protected with adjuvants that only induce CD8 immune responses. Clinical cases of listeriosis in 2013-2015 and mice sera have defined two useful prognostic immune biomarkers to design listeriosis vaccines: high anti-GAPDH antibody titers and tumor necrosis factor (TNF)/interleukin (IL)-6 ratios. Discussion: Gold glyco-nanoparticles vaccines conjugated to short LM peptides and formulated with a pro-inflammatory Toll-like receptor 2/4-targeted adjuvant protects safely against elderly and neonatal listeriosis. Mice vaccinated with these nanovaccines did not develop listeriosis or brain diseases and immune responses shifted towards Th1/IL-12 pro-inflammatory cytokine profiles and high production of anti-LM antibodies, suggesting good induction of LM-specific memory. Moreover, these nanovaccine formulations were able to activate Th1 production of monocyte dendritic cells from listeriosis patients, suggesting they might be a good nanovaccine formulation to implement LM-specific immunity in the elderly.

WS.E1.01 Visualizing immune responses

WS.E1.01.01

A hot trick for efficient peptide exchange on MHC class I multimers

J. J. Luimstra[1], *M. A. Garstka*[2], *M. C. Roex*[1], *F. H. Falkenburg*[1], *J. Neefjes*[1], *H. Ovaa*[1];
[1]*Leiden University Medical Center, Leiden, Netherlands,* [2]*Xi'an Jiaotong University, Xi'an, China.*

Fluorescently-labelled major histocompatibility complex class I (MHC I) multimers are widely used for the detection, isolation and analysis of T cells in infection, autoimmunity and cancer. We developed temperature-induced peptide exchange as a fast and flexible approach to generate large sets of MHC I multimers with different specificities in parallel. We designed conditional ligands for two dominant alleles, H-2K^b and HLA-A*02:01, that form stable complexes at 4°C, but dissociate from MHC I at a defined elevated temperature to be exchanged for a peptide of choice. We quantified the peptide exchange by HPLC and mass spectrometry and found that upon temperature exposure, the conditional ligand could efficiently be exchanged for both high- and low-affinity peptides. We performed peptide exchange on prefolded MHC multimers and used them directly to detect CD8$^+$ T cells responses to viral epitopes in mice infected with lymphocytic choriomeningitis virus or cytomegalovirus. We next put our multimers to clinical practice by monitoring the efficacy of adoptive T cell transfer in control of cytomegalovirus reactivation in an allogeneic stem cell transplant recipient. These data illustrate the flexibility and simplicity of using our temperature-exchangeable MHC I multimers. With this strongly improved multimer technology, high-throughput assessment of T cell responses in small sample sizes with become feasible. We will provide examples of this.

WS.E1.01.02

Chemokines and integrins independently tune actin flow and substrate friction during intranodal migration of T cells

M. Hons[1,2,3], *A. Kopf*[3], *R. Hauschild*[1], *A. Leithner*[1], *F. Gärtner*[1], *J. Abe*[2], *J. Renkawitz*[1], *J. V. Stein*[2], *M. Sixt*[1];
[1]*Institute of Science and Technology Austria, Klosterneuburg, Austria,* [2]*Theodor Kocher Institute, University of Bern, Bern, Switzerland,* [3]*Institute of Scientific Instruments of the Czech Academy of Sciences, Brno, Czech Republic.*

Although much is known about the physiological framework of T cell motility and numerous rate-limiting molecules have been identified in loss-of-function approaches, an integrating functional concept of T cell motility is lacking. Here we used *in vivo* precision morphometry together with analysis of cytoskeletal dynamics *in vitro* to deconstruct the basic mechanisms of T cell migration within lymphatic organs. We challenged the persisting concept that T cell locomotion consists of rounds of protrusive activity at the leading edge and retractions at the rear. We used intravital two-photon microscopy to show that T cells were faster the more they elongated but the actual change of the cell shape was unessential. Contributions of the integrin LFA-1 and the chemokine receptor CCR7 were complementary rather than positioned in a linear pathway, as they are during leukocyte extravasation from the blood vasculature. Our data demonstrate that CCR7 controlled speed of cortical actin flows, whereas integrins mediated substrate-friction that sufficed to drive locomotion in the absence of considerable surface-adhesions.

WS.E1.01.03

Inside the infected lung - Watching immune cells battle *Aspergillus fumigatus*

S. Henneberg[1], *S. Krappmann*[2], *L. Bornemann*[1], *M. Hasenberg*[1], *J. Weski*[1], *A. Hasenberg*[1], *M. Gunzer*[1];
[1]*Institute for Experimental Immunology and Imaging, University of Duisburg-Essen, University Hospital Essen, Essen, Germany,* [2]*Microbiology Institute – Clinical Microbiology, Immunology and Hygiene, University Hospital and Friedrich-Alexander-University of Erlangen-Nürnberg, Erlangen, Germany.*

Infection with the ubiquitous mold *Aspergillus fumigatus* is a life-threatening disease for immunosuppressed patients. Inhaled conidia can germinate in the lung and invade the tissue, which causes the invasive aspergillosis (IA). Because its diagnosis is time consuming and unreliable, IA is often diagnosed too late and the mortality rate reaches up to 95%. For the prevention of IA, a better understanding of the normally effective pulmonary immune response is essential. Since immune responses in the lung are regulated tightly by the microenvironment, the effect of an infection on immune cell functionality is best analysed *in vivo*.
Therefore, we established a novel intravital lung imaging technique that does not irritate the lung and maintains near physiological lung conditions during image acquisition by avoiding a pneumothorax. By triggering recordings at defined and short-time stable lung dilations prior to exhalation via a feedback of the ventilated lung, our advanced microscopy setup allows focus-stable documentation of immune cell behaviour during an infection. With a blood vessel staining and the usage of mouse lines with conditional expression of fluorescent proteins, we are able to study neutrophils and macrophages inside the lung tissue. First promising results exhibit changes of the recruitment, invasion, interaction and uptake of inhaled conidia of these immune cells. Our novel approach enables the visualisation of pulmonary immune cell function at almost physiological conditions in vivo.
This will open up new possibilities for the research and the understanding of pulmonary immunity and ultimately also the prevention and protection of dangerous lung diseases.

WS.E1.01.04

Imaging of cytotoxic antiviral immunity while considering the 3R principle of animal research

L. Otto[1,2]*, G. Zelinskyy*[1]*, M. Schuster*[2]*, U. Dittmer*[1]*, M. Gunzer*[2]*;*
[1]*Institute for Virology, Essen, Germany,* [2]*Institute for Experimental Immunology and Imaging, Essen, Germany.*

Adoptive cell transfer approaches for antigen-specific $CD8^+$ T cells are used widely to study their effector potential during infections or cancer. However, contemporary methodological adaptations regarding transferred cell numbers, advanced imaging, and the 3R principle of animal research have been largely omitted. Here, we introduce an improved cell transfer method that reduces the number of donor animals substantially and fulfills the requirements for intravital imaging under physiological conditions. For this, we analyzed the well-established Friend retrovirus (FV) mouse model. Donor mice that expressed a FV-specific T cell receptor (TCR^{tg}) and the fluorescent protein tdTomato were used as source of antigen-specific $CD8^+$ T cells. Only a few drops of peripheral blood were sufficient to isolate ~150,000 naive reporter cells from which 1000 were adoptively transferred into recently FV-infected recipients. The cells became activated and functional and expanded strongly in the spleen and bone marrow within 10 days post infection. Transferred $CD8^+$ T cells participated in the antiviral host response within a natural range and developed an effector phenotype indistinguishable from endogenous effector $CD8^+$ T cells. Additionally, the generated reporter cell frequency allowed single cell visualization and tracking of a physiological antiretroviral $CD8^+$ T cell response by intravital two-photon microscopy. Highly reproducible results were obtained in independent experiments by reusing the same donors repetitively for multiple transfers. Our approach allows a strong reduction of experimental animals required for studies on antigen-specific $CD8^+$ T cell function and should be applicable to other transfer models.

WS.E1.01.05

Visualisation of immune receptor sequences via projection into high-dimensional space with Siamese neural networks

V. I. Nazarov[1]*, E. Ofitserov*[2]*, V. Tsvetkov*[3,1]*;*
[1]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russian Federation,* [2]*Tula State University, Tula, Russian Federation,* [3]*Pirogov Russian National Research Medical University, Moscow, Russian Federation.*

Big data is often incomprehensible for humans and its visualisation is crucial for inference. Immunological data is no exception. TCR and immunoglobulin repertoires are commonly visualised with t-SNE technique or graphs. These approaches require a lot of resources to compute distance matrix - chiefly Levenshtein, which is also resource demanding - between sequences in repertoires. These obstacles significantly limit the amount of data algorithm can process in reasonable time. To overcome this issue a method for mapping immune receptor sequences into high-dimensional space was developed. The method relies on Siamese neural networks to map sequences and designed loss function to compare them in the new vector space. The latter estimates distances between vectors, which correlate with Levenshtein distance between corresponding sequences.
This approach facilitates data visualisation in terms of resources, especially computation time. Resulting receptor sequence embeddings can be used for further visualisation with t-SNE or UMAP algorithms. In order to evaluate the versatility of our approach the model was trained on 2 millions TCR sequences and applied to visualisation of 30 000 sequences via t-SNE. After visualisation the model appeared to conserve the information about Levenshtein distance between neighbours better than t-SNE applied to Levenshtein distance matrix. Moreover, the visualisation computational time was at least 30 times less than computation of Levenshtein distance matrix and consequent t-SNE visualisation. The benefit of this model is that once trained it is cheap in resources to make another computation and visualisation as it can be used in other contexts without any changes.

WS.E1.01.06

Intravascular resident Natural Killer cells shape neutrophil dynamics in the pulmonary vasculature

J. Secklehner[1,2]*, K. De Filippo*[2]*, J. B. Mackey*[1,2]*, J. Vuononvirta*[2]*, X. Raffo Iraolagoitia*[1]*, A. McFarlane*[1]*, M. Neilson*[1]*, M. B. Headley*[3]*, M. Krummel*[3]*, N. Guerra*[2]*, L. M. Carlin*[1,2]*;*
[1]*Cancer Research UK Beatson Institute, Glasgow, United Kingdom,* [2]*Imperial College, London, United Kingdom,* [3]*University of San Francisco, San Francisco, United States.*

Neutrophils are the most abundant leukocyte in the pulmonary vasculature, critical in the immune response to pathogens. The lungs' immune system must be finely-tuned to provide protection against pathogens while limiting tissue damage that could impede gas-exchange. Other immune cells play a role in controlling neutrophils in the lung (e.g. macrophages in initiating and resolving inflammation in the alveoli), however, intravascular neutrophil regulation in early acute lung inflammation may be pivotal for patient outcome. Natural Killer (NK) cells are enriched in the lung compared to other tissues and functional NK:neutrophil interactions have been described in several organs and conditions. We hypothesised that NK cells cooperate in controlling neutrophils in the pulmonary vasculature.
Lung intravital microscopy, to directly visualize the cells *in vivo,* revealed that NK cells can remain stationary for long periods (>60min) within alveolar capillaries, where neutrophils are able to move quickly. Furthermore, we found that nearly all NK cells in the lung are intravascular. NK cells and neutrophils frequently interact for 5-10 minutes in capillaries and occasionally material is transferred from neutrophils to NK cells. NK depletion resulted in marked changes to neutrophil dynamics such as track length and duration (48.77±6 to 112.2±15µm and 2.50±0.3 to 4.77±0.7min respectively). Stimulation with endotoxin in NK depleted mice lead to a more pronounced increase in neutrophil numbers compared to control mice. We propose that a population of intravascular resident lung NK cells directly regulates neutrophil responses to inflammation. Understanding how these cells interact could allow us to locally influence neutrophil behaviour.

WS.E2E3.01 Single cells to population dynamics and handling Big Data

WS.E2E3.01.01

MHC class II-omics for full understanding of MHC class II antigen presentation

M. L. Jongsma[1]*, R. Wijdeven*[1]*, B. Cabukusta*[1]*, D. Borras*[1]*, P. Paul*[2]*, T. van den Hoorn*[2]*, J. Neefjes*[1]*;*
[1]*LUMC, Leiden, Netherlands,* [2]*NKI, Amsterdam, Netherlands.*

MHC class II molecules present exogenously derived peptides to T lymphocytes facilitating immune responses and are strongly linked to autoimmune diseases. To get full insight into the cellular pathways controlling MHC class II antigen presentation, we generated and integrated datasets obtained from three flow cytometry-based genome-wide screens: 1) an RNAi-based screen, 2) a CRISPR-Cas9 knockout screen and 3) a CRISPR-Cas9 transcriptional activation screen. The analysis so far revealed a transcriptional network of nine regulators controlling MHC class II transcription as well as a GTPase controlled transport mechanism of MHC class II containing compartments (MIIC) in human dendritic cells. We will present data on new routes and pathways in control of MHC class II antigen presentation. This will yield new leads for manipulating the immune system in the case of autoimmune diseases, infections and tumor immunology.

WS.E2E3.01.02

High affinity T cell receptor generate both active and memory cytotoxic response during a viral infection.

F. Luciani[1]*, S. Rizzetto*[1]*, A. Eltahla*[1]*, C. Cai*[1]*, E. Keoshkerian*[2]*, K. Wing*[2]*, D. Koppstein*[2]*, W. Van der Byl*[1]*, K. Shober*[3]*, M. Effemberger*[4]*, R. Bull*[1]*, A. R. Lloyd*[2]*, D. Busch*[3]*;*
[1]*School of Medical Sciences, Sydney, Australia,* [2]*Kirby Institute, Sydney, Australia,* [3]*Technical University Munich, Munich, Germany,* [4]*Technical University Munich, Munics, Germany.*

Cytotoxic CD8 T cells (CTL) play a key role in controlling viral infection by killing infected cells and establishing protective immunological memory. The affinity and diversity of T cell receptor (TCR) are critical factors that drive the activation and differentiation of CTL, but the molecular mechanisms underlying these dynamics remains elusive. We developed a novel single cell approach to measure and link TCR affinity, gene profiles using single-cell (sc)RNAseq,and functional status of CTL.We analysed >1000 antigen specific (Ag) CD8 T cells using autologous tetramers derived from longitudinal samples of early-phase HCV infections. By using VDJPuzzle, a bioinformatics pipeline to reconstruct full-length TCR from scRNAseq we surprisingly discovered in one subject a monoclonal CD8 T-cell response targeting an HLA-I-B07 restricted peptide (GPR) with high IFN-γ(1600 SFU/10^6cells) induction and a highly activated phenotype. Reversible streptamer technology revealed a high-affinity (K_{off}=8.1x10^{-4} sec^{-1})TCR for GPR peptide whilst scRNAseq, showed multiple subsets with distinct gene profiles in the GPR response. This included a rare memory precursor subset ($CD127^+KLRG1^-$) with a novel gene signature and a metabolic profile consistent with a resting state, oxidative phosphorylation and fatty acid metabolism. In contrast, a second polyclonal response targeting an HLA-IA01 restricted-peptide (ATD) was also found in the same subject, which by contrast generated low IFN-γand carried gene signatures distinct from the GPR-CTL response. The results suggest that the strength of TCR binding to cognate Ag shape the phenotype and kinetics of Ag specific CD8 T cell response. These findings are currently being validated on more subjects.

WS.E2E3.01.03

A Semi-Unsupervised Multivariate Algorithm for Mining and profiling High-dimensional Flow Cytometry Data

P. C. Martins Urbano[1], *L. Rosaria*[2], *W. Alkema*[2], *A. Koerber*[3], *E. de Jong*[4], *F. Hoentjen*[5], *I. Joosten*[1], *H. J. Koenen*[1];
[1]*Radboud university medical center, Department of Laboratory Medical Immunology, Nijmegen, Netherlands,* [2]*Radboud university medical center, Centre for Molecular and Biomolecular Informatics, Nijmegen, Netherlands,* [3]*University Hospital Essen, Department of Dermatology, Essen, Germany,* [4]*Radboud university medical center, Department of Dermatology, Nijmegen, Netherlands,* [5]*Radboud university medical center, Department of Gastroenterology and Hepatology, Nijmegen, Netherlands.*

Introduction: Flow cytometry is an important technology for the diagnosis of life threatening diseases and has great potential for immune profiling of immune mediated inflammatory diseases (IMIDs). The current definition of diseases is largely based on 14th century medicine that is mostly based on the clinical manifestations but not on complex cellular and molecular interactions underlying disease pathology. Flow cytometry might provide IMID associated immune profiles that support diseases stratification, efficient therapy selection and therapy monitoring. Here we used flow cytometry combined with a semi-unsupervised multivariate algorithm and a machine learning tool to generate immune profiles in order to classify a variety of IMIDs.
Materials: Whole blood and peripheral blood mononuclear cells (PBMCs) from healthy controls, psoriatic (Ps), atopic dermatitis (AD) and inflammatory bowel disease (IBD) patients were collected and stained with five 10-color flow cytometry panels (1.General adaptive/innate cells, 2. T-cell differentiation/maturation, 3. B-cell differentiation/maturation, 4.Thelper cells and 5.Regulatory Tcells), measured by flow cytometry, followed by manual data analysis and subsequent unsupervised multivariate analysis and data mining by random forest machine learning.
Results: The multivariate algorithm combined with the machine learning tool are capable to stratify the IMID cohorts and the healthy volunteer cohort. Random forest analysis identified of several immune cell subsets that distinguish between those disease cohorts. However, many common denominators were found between PS, AD, and IBD.
Conclusion: We developed a semi-unsupervised analysis pipeline to profile and mine high-dimensional flow cytometry data that might be applied for IMID stratification.

WS.E2E3.01.04

Single cell transcriptomes define the cellular and molecular landscape of human lungs in asthma

F. Vieira Braga[1], *G. Kar*[1], *T. Gomes*[1], *E. S. Fasouli*[1], *P. Strzelecka*[1], *K. Polanski*[1], *M. Efremova*[1], *K. Mahbubani*[2], *A. Cvejic*[1], *K. Saeb-Parsy*[2], *O. A. Carpaij*[3], *M. Berg*[3], *S. Brouwer*[3], *K. Affleck*[4], *M. van den Berge*[3], *A. van-Oosterhout*[4], *M. Nawijn*[3], *S. A. Teichmann*[1];
[1]*Wellcome Trust Sanger Institute, Cambridge, United Kingdom,* [2]*Cambridge University, Cambridge, United Kingdom,* [3]*University of Groningen, Groningen, Netherlands,* [4]*GlaxoSmithKline, Stevenage, United Kingdom.*

The lung plays a critical role in both gas exchange and mucosal immunity. Acute and chronic disorders of the lung are a major cause of mortality worldwide, and some are expressed predominantly in the airways (such as asthma) or in the respiratory unit in lung parenchyma (such as emphysema). We comprehensively profiled the cells that make up both the human lung upper airway wall and the respiratory unit, as well as paired spleens and lymph nodes using single cell transcriptomics. We identified core signatures of lung resident immune cells in healthy individuals. We expanded our analysis beyond healthy individuals by performing single cell transcriptome analysis of whole airways biopsies and sorted CD4 T cells from asthmatic patients. We observed disease specific programs of epithelial cell differentiation and reshaping of the immune repertoire that takes place in asthma. We reconstructed cellular communication networks between immune and non immune cells specific for asthma. Our data constitute an invaluable resource for the community interested in immune responses localised to the lung.

WS.E2E3.01.05

DiSNE visualization and assessment of clonal kinetics reveals multiple trajectories of dendritic cell development

D. Lin[1], *A. Kan*[1], *J. Gao*[1], *E. Crampin*[2], *P. Hodgkin*[1], *S. Naik*[1];
[1]*Walter and Eliza Hall Institute, Melbourne, Australia,* [2]*University of Melbourne, Melbourne, Australia.*

A thorough understanding of cellular development is incumbent on assessing the complexities of fate and kinetics of individual clones within a population. Here, we develop a system for robust periodical assessment of lineage outputs of thousands of transient clones and establishment of *bona fide* cellular trajectories. We appraise the development of dendritic cells (DCs) in *fms*-like tyrosine kinase 3 ligand culture from barcode-labeled hematopoietic stem and progenitor cells (HSPCs) by serially measuring barcode signatures, and visualize this multidimensional data using developmental interpolated t-distributed stochastic neighborhood embedding (Di-SNE) time-lapse movies. We identify multiple cellular trajectories of DC development that are characterized by distinct fate bias and expansion kinetics, and determine that these are intrinsically programmed. We demonstrate that conventional DC and plasmacytoid DC trajectories are largely separated already at the HSPC stage. This framework allows systematic evaluation of clonal dynamics and can be applied to other steady-state or perturbed developmental systems.

WS.E2E3.01.06

CD4 T cell transcriptomics reveals novel diagnostic and mechanistic immune signatures of tuberculosis

J. G. Burel[1], *C. S. Lindestam Arlehamn*[1], *M. Pomaznoy*[1], *N. Khaan*[1], *G. Seumois*[1], *J. A. Greenbaum*[1], *D. DeSilva*[2], *R. Taplitz*[3], *R. H. Gilman*[4], *M. Saito*[5], *P. Vijayanand*[1], *A. Sette*[1], *B. Peters*[1];
[1]*La Jolla Institute for Allergy and Immunology, La Jolla, United States,* [2]*General Sir John Kotelawala Defence University, Colombo, Sri Lanka,* [3]*University of California San Diego, La Jolla, United States,* [4]*Johns Hopkins University Bloomberg School of Public Health, Baltimore, United States,* [5]*Tohoku University, Sendai, Japan.*

In the context of infectious diseases, cell population transcriptomics are useful to gain deeper insight into protective immune responses, which is limited using traditional whole blood approaches. As part of the Human Immune Project Consortium (HIPC) program, we are aiming to decipher CD4 T cell immune signatures of tuberculosis (TB) in the context of controlled (latent TB) and uncontrolled (active TB) infection. We found an *ex vivo* gene expression signature in memory CD4 T cells that could differentiate between latent TB and TB negative subjects, as well as novel markers that are expressed in TB-antigen specific CD4 T cells in the context of latent TB. We further explored the inter-individual variability of gene expression within the latent TB cohort and identified immune parameters associated with higher risk of developing active TB. Finally, comparison of the transcriptomic profile of memory CD4 T cells of latent and active TB subjects after antigen-specific *in vitro* stimulation identified TB-specific T cell immune signatures of controlled infection versus disease. Overall, our approach has identified a plethora of TB-associated T cell immune signatures that allowed us to gain mechanistic insights into the key CD4 T cell components contributing to TB protective immunity. Additionally, our findings can be translated into new diagnostic and prognostic tools, in particular, identifying latent TB infected individuals at risk of developing active TB.

WS.E4.01 Cell communication and signaling in the immune system

WS.E4.01.01

Mechanotransduction as a novel immune checkpoint in NK cell cytotoxicity

M. Barda-Saad, *A. Ben-Shmuel, O. Matalon, J. Kivelevitz, B. Sabag, N. Joseph, G. Biber;*
Bar-Ilan University, Ramat-Gan, Israel.

Natural killer (NK) cells are a potent weapon of the immune system against viral infections and tumor growth. The actomyosin network generates forces through the activity of actin filaments and myosin motors. This machinery is responsible for the conversion of mechanical forces into biochemical signals in a process termed mechanotransduction. However, the mechanism by which mechanotransduction controls the immune response, and specifically lymphocyte activity, is poorly understood. Here, we demonstrate that actomyosin retrograde flow (ARF) controls NK cell response through a novel interaction between beta-actin with the SH2-domain containing protein tyrosine phosphatase-1 (SHP-1), converting its conformation state, thereby regulating NK cell cytotoxicity. Actin dynamics govern SHP-1 conformational structure dictating its catalytic activity. Indeed, blocking actin dynamics results in reduced SHP-1 activity, by confining SHP-1 to its inactivated “closed” conformation. This reduced enzymatic activity of SHP-1 leads to increased phosphorylation of SHP-1 substrates, an elevation of intracellular calcium flux, and NK cell cytotoxicity. Our data suggest that SHP-1 plays a major role as a sensor of ARF-generated forces in the process of mechanotransduction, and reveal a novel mechanism by which regulation of SHP-1 by ARF dictates NK cell killing decisions. Our data identify ARF as a master regulator of the lymphocyte response.

WS.E4.01.02

Combining FACS, High-Resolution Mass-spectrometry, and Single Cell Sequencing Reveals Interactome Effects of Translational Control

M. D. Stadnisky, *R. Halpert, S. Siddiq, J. Spidlen, J. Almarode, M. Velazquez-Palafox, I. Taylor, C. Freier;*
FlowJo, LLC & Becton, Dickinson and Company, Ashland, United States.

Algorithms can target advertisements based on volunteered content and social network in microseconds, yet precision medicine lags in infancy. To break this societal Munchausen by proxy, we generated a framework for immune health by creating a social network of immune cells, modeling both internal state and interactions with other cells. To do this, we analyze and combine data sets including (1) FACS with high-resolution mass-spectrometry-based proteomics and (2) more than 20 single cell whole transcriptome datasets. With (truly) quantitative protein and gene expression data for 28 human hematopoietic cell populations, and more than 10,000 proteins and genes measurements per cell, we generate an interactome for these cells and layer in both transcriptional and translational controls. Using these as internal 'sentiments', we develop a deep steady state social network such that perturbations to the internal state of individual cells (e.g. in the context of disease) can be modeled.
Using three examples, (1) CD8 T cell exhaustion, (2) alternate macrophage activation, and (3) dendritic cell dysfunction, we show how perturbations in immune cell gene and protein expression affect the network of immune cells, and ultimately the immune system. Finally, we propose a set of assays to establish a patient steady state profile, and we describe how to build a precision, predictive social network for an individual's immune system.

WS.E4.01.03

Helper T cell extracellular vesicles couple delivery of effector CD40L to antigen recognition

E. B. Compeer[1], *D. G. Saliba*[1], *P. F. Céspedes*[1], *Š. Bálint*[1], *K. Korobchevskaya*[1], *C. Cassioli*[2], *M. L. Dustin*[1];
[1]*University of Oxford, Oxford, United Kingdom,* [2]*University of Siena, Siena, Italy.*

Most, if not all, cell types release extracellular vesicles (EV) that play a role in local and systemic intercellular communication by transferring protein, lipids and RNA between cells. Exosomes, a subset of EVs, arise through exocytosis of multivesicular bodies that lead to release of their intraluminal vesicles, which are mostly formed by vesicle budding and scission from endosomal membranes by the Endosomal Sorting Complexes Required for Transport (ESCRT). We recently discovered a parallel route of EV generation in CD4 T-cells. We found that >80% of TCR at the immunological synapses' center, formed with supported planar bilayers (SLB) containing peptide-MHCII and ICAM1, are released from the T-cell surface. These EV thus can be defined as synaptic ectosomes (SE) based on budding from the plasma membrane.
Mass spectrometry analysis of SE demonstrates the presence of TCR signaling and ESCRT proteins, corroborating our earlier data. While CD40L was not normally detected in SE we could recruit CD40L to SE by incorporating CD40 into SLBs. Super-resolution microscopy on SLBs shows that the majority of SE are TCR positive, with half of the TCR T-EV positive for CD40L, in presence of TCR and CD40 engagement. Exposure of human dendritic cells (DC) to CD40L-SE induced DC maturation. We are investigating the form of CD40L that is stored and translocated in T-cells for packaging and release with TCR at the IS resulting in a feed forward activation of DC with potential for intrinsic peptide-MHC specificity.

WS.E4.01.04

Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1

D. G. Ryan;
Trinity Biomedical Sciences Institute, Dublin, Ireland.

The endogenous metabolite itaconate has recently emerged as a regulator of macrophage function. However, the precise mechanism of itaconate action in macrophages remains poorly understood. Here we report that itaconate is required for the activation of the anti-inflammatory transcription factor nuclear factor-erythroid 2 p45-related factor 2 (Nrf2) by LPS. We find that itaconate directly modifies proteins via alkylation of cysteine residues, a novel post-translational modification we've termed 2,3-dicarboxypropylation. Itaconate alkylates cysteines 151, 257, 273, 288 and 297 on the Kelch-Like ECH-Associated Protein 1 (Keap1) enabling Nrf2 to increase expression of downstream genes with antioxidant and anti-inflammatory capacity. The activation of Nrf2 is required for the anti-inflammatory action of itaconate. We describe the use of a new cell-permeable itaconate derivative, 4-octyl itaconate (OI), which is protective against LPS-induced lethality *in vivo* and decreases cytokine production. We show that type I interferons (IFN) boost immunoresponsive gene 1 (*Irg1*) expression and itaconate production. Furthermore, we find that itaconate production limits the type I IFN response indicating a negative feedback loop involving IFNs and itaconate. Our findings demonstrate that itaconate is a critical anti-inflammatory metabolite acting via Nrf2 to limit inflammation and modulate type I IFNs.

WS.E4.01.05

Sugar sweet signalling; Exploration of dendritic cell lectin receptors and their immune modulatory signalling pathways

R. J. E. Li, *J. E. Rodriguez-Camejo, J. Lübbers, A. Zaal, C. R. Jimenez, S. R. Piersma, T. V. Pham, R. R. de Goeij- de Haas, S. J. van Vliet, Y. van Kooyk;*
Cancer Center Amsterdam - VU University medical center, Amsterdam, Netherlands.

Dendritic cells (DCs) are key inducers of the adaptive immune response. They possess a multitude of different pattern recognition receptors (PPRs), including Toll-like receptors (TLRs), and C-type Lectin Receptors (CLRs) in order to elicit tailor-made immune response against invading pathogens. CLRs have gained much attention as endocytic PRRs, but also for their immune modulatory functions. Strikingly, several carbohydrate ligands are shared among different CLRs, yet each seems to propagate a unique signalling cascade. Additionally, CLR triggering can modulate the signalling pathways of TLRS, to modify or prolong the TLR-induced response.
We recently showed that carbohydrates have a huge impact on DC polarizion or suppression of T cell responses. Carbohydrates such as High Mannose-, LewisX-, or LewisY-containing ligands displayed differential IL-10 and IL-12 expression profiles by DC after concomitant LPS stimulation. In contrast, α2-3- or α2-6-sialic acid-containing ligands skew DCs towards the induction of T regs. These carbohydrates target differential CLRs such as DC-SIGN and Siglec, respectively. To gain further insight the immunogenic signalling pathways of the DC-expressed CLR DC-SIGN and its interference with TLR signalling, as well as the tolerogenic signalling pathways through Siglec, we coupled different carbohydrate ligands to a dendrimeric structure, thereby offering multivalent ligand presentation. For insight in the underlying signalling pathways, we applied phosphoproteomics to investigate differences in DC protein phosphorylation upon specific CLR-ligand engagement, and next generation sequencing on a transcriptional level. Detailed analysis of these reveal an immunogenic or tolerogenic DC fingerprint through carbohydrate-CLR interaction, and include significant changes in immunogenic signalling pathways.

WS.E4.01.06

DLL4 conveys Notch-dependent signals achieving selective macrophage polarization or death

B. CHARREAU[1], *S. Pagie*[1], *A. Pabois*[1], *N. Gérard*[1], *C. Toquet*[1], *P. Hulin*[2], *S. Nedellec*[2];
[1]*CRTI, Nantes, France,* [2]*Plateforme MicroPICell SFR Santé –IRT, Nantes, France.*

1Molecular mechanisms underlying vascular and inflammatory cell network at endothelial and macrophage levels are still unclear. Here we found that microvascular inflammation associates with changes in Notch signaling at endothelium/monocyte interface including loss of endothelial Notch4 and the acquisition of the Notch ligand Dll4 in both cell types.
We showed that endothelial DLL4 induces circulating monocytes to polarize into a M1-type pro-inflammatory macrophages ($CD40^{high}CD64^{high}CD200R^{low}$ HLA-$DR^{low}CD11b^{low}$) eliciting the production of IL-6. Dll4 and IL-6 are both Notch-dependent and are required for macrophage polarization through selective down and upregulation of M2- and M1-type markers, respectively. Subsequently, we investigated the ability of DLL4 to interfere with M2 polarization. We found that DLL4 triggers a specific alteration of the IL-4 induced M2 phenotype through a significant inhibition of M2 markers (CD11b, CD206, CD200R). DLL4 also induces caspase3/7-dependent apoptosis specifically in M2 differentiating macrophages while DLL1 had no effect. DLL4 signals via Notch1 and DLL4-mediated apoptosis is Notch-dependent. Fully differentiated M2 macrophages became resistant to DLL4 action**.** DLL4 upregulates gene expression, upon M2 upon differentiation, affecting the Notch pattern (Notch1, 3, Jag1) and activity (Hes1), transcription (IRF5, STAT1) that associates with decrease in Akt but not STAT6 phosphorylation. In conclusion, our findings reveal an interplay between DLL4/Notch and IL-6/IL-6R or IL-4/IL-4R signaling pathways supporting M1 differentiation and impairing M2 differentiation via apoptosis.

POSTER PRESENTATIONS

P.A1.01 Myeloid lineage specification - Part 1

P.A1.01.01

Advancing knowledge on human dendritic cell types and activation states through the design and study of in vitro differentiation models.

*S. Balan[1,2], C. Arnold-Schrauf[1], A. Abbas[1], N. Couespel[1], J. Savoret[1], F. Imperatore[1], A. Villani[3], T. Vu Manh[1], N. Bhardwaj[2], **M. Dalod**[1,4];*
[1]Aix Marseille Univ, CNRS, INSERM, CIML, Marseille, France, [2]2The Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, United States, [3]3Broad Institute of Harvard University and MIT,, Cambridge, United States, [4]Centre d'Immunologie de Marseille-Luminy (CIML), Marseille, France.

Our understanding of the biology of human dendritic cells and our ability to harness them clinically are hampered by lack of adequate in vitro models of these cells. Ideally, such in vitro models should combing high yields of different types of dendritic cells in the same culture and their amenability to genetic or pharmacological manipulation. To overcome this bottleneck, we developed a method of differentiation of human CD34+ precursors leading to high yields of all the three major types of human blood dendritic cells, namely plasmacytoid dendritic cells, type 1 (CD141-positive) conventional dendritic cells and type 2 (CD1c-positive) conventional dendritic cells. Phenotypic, functional and single cell RNA sequencing analyses were performed to ensure of the identity of the dendritic cell types derived in vitro and of their strong homology with their ex vivo isolated counterparts. This culture system also revealed novel molecular mechanisms governing the differentiation and/or expansion of human dendritic cell types which will be discussed in this presentation. Combining several such recent novel in vitro models for the differentiation of distinct types of human mononuclear phagocytes will greatly facilitate the simultaneous and comprehensive study of primary, otherwise rare, types of human dendritic cells or macrophages, including their mutual interactions.

P.A1.01.02

GM-CSF and M-CSF signaling is integrated at chromatin level during human monocyte polarization

*R. M. Rodriguez[1], B. Suárez Álvarez[1], **P. Díaz Bulnes**[1], J. L. Lavín[2], A. M. Ascensión[3], M. Gonzalez[2], A. Baragaño Raneros[1], C. Martin-Martin[1], A. Puig-Kröger[4], A. L. Corbí[5], M. J. Araúzo-Bravo[3], A. M. Aransay[2], C. López Larrea[1];*
[1]Traslational Immunology Laboratory, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA); Hospital Universitario Central de Asturias (HUCA), Oviedo, Spain, [2]BioGUNE, Bizkaia, Spain, [3]Computational Biology and Systems Biomedicine Research Group, Biodonostia Health Research Institute, San Sebastián, Spain, [4]Laboratorio de Inmuno-Metabolismo, Hospital General Universitario Gregorio Marañón, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain, [5]Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain.

Over the last few years, myelopoietic growth factor signaling has emerged as an essential player during monocyte polarization. Due to the apparent antagonism between GM-CSF and M-CSF and their central role during monocyte differentiation, both factors have been proposed as suitable therapeutic targets. Nonetheless, since both cytokines can be simultaneously present in the inflamed tissue it is not clear how signaling is integrated during cell fate decisions or whether monocytes are able to fully reverse their phenotypes in response to apparently conflicting polarization cues. Previous studies showed that a preformed chromatin state generated during differentiation or in response to a given stimulus can influence how subsequence activation cues modify gene expression, indicating that the chromatin acts as an integration node during monocyte cell fate decision in the inflammation site. To better understand how undifferentiated monocytes integrate GM-/M-CSF signaling at the epigenetic level we performed DNA methylation analysis and ATAC-seq in *ex vivo* isolated human monocytes. Our results revealed a global and irreversible demethylation trend associated with M-CSF and GM-CSF exposure. In addition, chromatin accessibility analysis showed an extensive epigenetic remodeling after only 12 hours exposure in genes typically associated with monocyte polarization. Finally, by specific inhibition of the canonical GM-CSF receptor signaling modules, we were able to dissect the epigenetic changes associated with the JAK-dependent (STAT5, PI3K and MAPK) and JAK-independent signaling pathways. In summary, our results indicate that M-CSF and GM-CSF signaling is rapidly integrated at chromatin level in order to generate stable gene expression patterns during monocyte differentiation.

P.A1.01.03

Identity of human lymphoid organ dendritic cells is predominantly dictated by ontogeny, not tissue microenvironment

*G. F. Heidkamp[1,2], J. Sander[3], C. H. Lehmann[1,2], L. Heger[1,2], A. Baranska[1,2], J. J. Lühr[1,2], A. Hoffmann[1,2], K. C. Reimer[1,2], A. Lux[1], A. Hartmann[1], T. Ulas[3], N. McGovern[4], C. Alexiou[1], B. Spriewald[1], A. Mackensen[1], G. Schuler[1], R. Repp[5], P. A. Fasching[1], R. Cesnjevar[1], E. Ullrich[6], F. Ginhoux[4], A. Schlitzer[3], F. Nimmerjahn[1], J. L. Schultze[3], **D. Dudziak**[1,2];*
[1]Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen, Germany, [2]Laboratory of DC-Biology, Erlangen, Germany, [3]LIMES, Bonn, Germany, [4]Singapore Immunology Network, Singapore, Singapore, [5]Städtisches Krankenhaus Kiel, Kiel, Germany, [6]Hospital of the Goethe University, Frankfurt, Germany.

Dendritic Cells (DCs) are important regulators of immune responses. So far, the distinct functional roles of human tissue DC subpopulations remain largely unknown. In the here presented study, we directly isolated DC subpopulations from various human lympho-hematopoietic tissues (thymus, spleen, bone marrow, tonsils, cord blood, peripheral blood). We performed up to 20-paramter FACS analyses for the study of 230 cell surface molecules. We found that the percentages of the three main DC subpopulations of $CD1c^+$ DCs, $CD141^+$ DCs, and pDCs were varying depending on the analyzed tissue. We next investigated the transcriptional profile of cell-sorted DC subpopulations using comprehensive bio-informatic data analyses. Our data are supported by investigation of DC subset localization and functional studies. Overall, we conclude that human DCs of the lympho-hematopoietic system are mainly defined by ontogeny, while non-lympho-hematopoietic DCs (lung, skin) seem to be additionally influenced by modulatory signals from the tissue microenvironment.
The here presented data will help to elucidate the implication of human DC subpopulations in the initiation of immune responses.
This work was partly supported by DFG (Emmy-Noether Program, CRC643, CRC1181, CRC645, CRC704, RTG1660, RTG1962, SPP1681), Loewe Center, ELAN, IZKF, BayGene, and Excellence Cluster ImmunoSensation.

P.A1.01.04

Dendritic cells modulation by mesenchymal stem cell promises a protective microenvirment at the feto-maternal interface: improved outcome of pregnancy in abortion prone mice

***M. Eskandarian**, A. Yaftiyan, A. Jahangiri, S. Moazzeni;*
Tarbiat modares university, Tehran, Iran, Islamic Republic of.

Introduction: Recurrent spontaneous abortion is one of the most common complications of pregnancy. A major fraction of RSA is related to disorder of the maternal immune system, especially malfunction of dendritic cells (DCs). MSCs have been shown to exert immunomodulatory effects on immune cells especially dendritic cells. The current study investigates whether MSCs are capable to modulate the pattern of maternal immune response via the induction of functional changes in decidual DCs, and finally improves the fetal survival.
Materials and Methods: For this issue, adipose derived mesenchymal stem cells were intraperitonealy administered to abortion prone pregnant mice (CBA/J ×DBA/2) at fourth day of gestation. On day 14.5 of gestation, after determination of abortion rates, the number, phenotype and maturation state of decidual dendritic cells were analyzed using the flow cytometry.
Results: We found that MSCs therapy could decrease the abortion rate significantly and at the same time increases the frequency of decidual DCs. MSCs administration also remarkably decreased the expression of MHC-II, CD86 and CD40 markers on decidual DCs in MSC-treated group. In contrast, CD11b significantly increased in these group compared to non-treated mice.
Conclusions: Our results indicated that MSCs could change the microenvironment of decidua through secretion of various components or direct cell-cell contact and correct the immune cell disorders by modulating the DCs function.

P.A1.01.05

Gene scan analysisby detection of GTdel in exon 2in NCF1 gene for AR-CGD with p47 defect

***M. Y. Koker**, B. Saraymen;*
Erciyes Medical school, Kayseri, Turkey.

CGD patients have 5 different gene which is most popular for genetic mutations. But in patients with residual oxidase activity in DHR assay mostly have mutation in NCF1 gene defect result in p47-phox defect. Most of mutation in CGD and NCF1 gene are result from hot-spot mutation(C.75_76delGT) in exon which results in p.[Try26HisFx26]. One of the fastest method for detection of mutation in these patients are gene scan analyisis by using microstellite analysis for detection of pseudogene/gene ratio in NCF1 gene. When the gene scan peak ratio is 1:1, usually this means that one allele there is the normal cofiguration of two pseudogenes and one NCF1 gene. On the other allele we then find one pseudogene, one NCF1 gene and one fusion gene of the 5'part NCF1 gene and the 3'part pseudogene. The gene scan detects the 5'part of the pseudogenes and the NCF1 genes. That leads in such a case to three genes with the deltaGT (pseudogene) and three genes with the GTGT configuration (like in NCF1 genes). Thus, the ratio is 3:3 = 1:1. When the fusion genes are inactive, so you can regards this as a heterozygote for NCF1 deltaGT. In such a person, there is a no defect in oxidase activity, p47 expression or microbicidal activity. We have dizayn a gene-scan diagram first time in the literatüre.

P.A1.01.06

Resident macrophages in the skin interconnect immune and nervous system

J. Kolter[1,2], *R. Feuerstein*[1], *N. Hagemeyer*[3], *P. Zeis*[4], *N. Paterson*[4], *D. Grün*[4], *T. Lämmermann*[4], *M. Prinz*[3], *P. Henneke*[1];
[1]*Center for Chronic Immunodeficiency, Freiburg, Germany,* [2]*Faculty of Biology, University of Freiburg, Freiburg, Germany,* [3]*Institute of Neuropathology, Freiburg, Germany,* [4]*Max-Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany.*

Since dermal macrophages but not monocytes are central for the defense against *Staphylococcus aureus*, we reasoned that heterogeneous mechanisms of macrophage replacement coexist in the skin. Here, we scrutinized the origin of dermal macrophages and a potential postnatal specification into subpopulations by utilization of reporter mice, fate-mapping strategies and transcriptomics.
We found that dermal macrophages underwent fundamental changes after birth. While the population initially uniformly expressed the fractalkine receptor at high level (CX_3CR1^{bright}), only a small fraction remained positive in adult mice. CX_3CR1^{bright} macrophages were only slowly replaced after bone marrow transfer and exhibited long persistence after tamoxifen-dependent fate mapping in *Cx$_3$cr1$^{creER/+}$:R26-yfp* mice. In contrast, other dermal macrophages were short-lived and received major bone-marrow input. Single cell RNA sequencing revealed six major macrophage clusters in the skin of which one corresponded to the CX_3CR1^{bright} population. The respective subset closely and exclusively colocalized to sensory nerve axons in the dermis of adult mice and showed a distinct expression signature with over 500 upregulated genes including anti-apoptotic and migratory markers in bulk transcriptomic analysis. Specific expression of markers connected to the nervous system along with bidirectional migration along nerve axons and uptake of myelin point towards a role interconnecting immune and nervous system, e.g. in the context of inflammatory nerve damage.
Collectively, these data point to a sub-specification of skin macrophages leading to heterogeneity in phenotype, function and origin within the very same tissue.

P.A1.01.07

Defining the role of E-cadherin in hematopoiesis

R. A. Krimpenfort*, M. Nethe;*
Sanquin Blood Supply, Amsterdam, Netherlands.

During erythropoiesis red blood cells (RBCs) are generated to adsorb and transport oxygen which is a fundamental process to sustain life. The generation of RBC's importantly relies on stem cells in the bone marrow which produce RBC's during erythropoiesis. During erythropoiesis erythroblasts, precursors of RBC's, mature into RBCs in the close proximity of a central macrophage which is hypothesized to control erythropoiesis. Little, however is known about the mechanism by which these erythroblasts adhere and detach from this central macrophage and how these macrophages allow maturation and formation of erythrocytes. Intriguingly, the cell adhesion protein E-cadherin, which expression is in general restricted to epithelial cells has been indicated to be expressed in the erythroid cell lineage. Moreover, induction of E-cadherin expression marks myelodysplastic syndrome (MDS) which is an age-related bone marrow malignancy characterized by dysplastic lesions in the bone marrow and a hampered production of myeloid cells. MDS often develops into acute myeloid leukemia (AML) and is therefore in general viewed to display precursors of AML. Since E-cadherin functions as an important tumor suppressor in epithelial tissue it questions to what extend E-cadherin similarly can acts as a tumor suppressor during development of blood-borne cancers like AML. E-cadherin expression is indeed frequently silenced by promoter hypermethylation in AML. The aim of this study is therefore to dissect the role of E-cadherin in hematopoiesis and development of myeloid malignancies by using a novel mouse model which allows somatic inactivation of E-cadherin in the hematopoietic cell lineage.

P.A1.01.09

Selective expansion of lineage-primed progenitors guides emergency haematopoiesis

D. Lin*, P. Hodgkin, S. Naik;*
Walter and Eliza Hall Institute, Melbourne, Australia.

Single hematopoietic stem and progenitor cells (HSPCs) are demonstrated to be lineage-primed in the steady state using clonal fate tracking and single cell RNA profiling. However, how single cell fate is regulated during emergency conditions that lead to skewing of lineage production is largely unknown. Here, we tag individual HSPCs with unique and heritable DNA barcodes and track their fate *in vivo* under Fms-like tyrosine kinase 3 (Flt3) ligand (FL) induced emergency state, which mediates a specific increase in dendritic cell (DC) numbers. We observe dramatic clonal expansion of HSPCs that accounts for the majority of emergency DC generation, with more profound contribution from multipotent clones than those with restricted fate. Importantly, such expansion is selective towards the DC lineage, as production of other lineages remain unchanged at the clonal level.
In contrast, we observe minimal contribution by recruitment of other clones. Collectively, our results suggest that excess FL guides emergency DC development by selectively enhancing proliferation and differentiation of pre-existing progenitors along the DC trajectory, without diverging fate from non-DC primed progenitors. Thus, this suggests that lineage program within single HSPCs are already stably established and fate 'plasticity' via extrinsic regulation is not the major mechanism. These findings provide new insight into the control and regulation of fate during hematopoiesis.

P.A1.01.11

Investigation of BATF-dependent gene expression signatures in plasmacytoid dendritic cells

R. Mann-Nüttel[1], *S. Ali*[1,2], *P. Petzsch*[3], *K. Köhrer*[3], *H. Xu*[4], *P. Lang*[4], *S. Scheu*[1];
[1]*Institute of Medicinal Microbiology and Hospital Hygiene, Heinrich Heine University Düsseldorf, Düsseldorf, Germany,* [2]*Cluster of Excellence EXC 1003, Cells in Motion, University of Münster, Münster, Germany,* [3]*Biomedical Research Facility, Heinrich Heine University Düsseldorf, Düsseldorf, Germany,* [4]*Institute of Molecular Medicine, Heinrich Heine University Düsseldorf, Düsseldorf, Germany.*

Plasmacytoid dendritic cells (pDCs) are known to produce large amounts of type I interferon (IFN) during viral infections. Contradicting the previous dogma, we found that only a small subpopulation of pDCs produces type I IFN after TLR9 activation. Microarray experiments revealed that the transcription factor *Batf* (basic leucine zipper ATF-like transcription factor) is highly expressed in these type I IFN-producing pDCs. While the function of BATF for T helper cell subset differentiation and B cell class switching has been well described, no biological role of BATF in pDCs has been shown so far.
Analyzing *Batf*$^{-/-}$ mice and wildtype (WT) littermates we found an increased frequency of pDCs in Flt3-L cultures from bone marrow (BM) of *Batf*$^{-/-}$ mice. While splenic pDCs from *Batf*$^{-/-}$ mice and WT controls expressed equivalent levels of MHC class II and costimulatory molecules, surface levels of the canonical DC marker CD11c were markedly reduced in *Batf*$^{-/-}$ pDCs. Further, analyzing the expression of key transcription factors involved in DC development in pDCs derived from Flt3-L BM cultures and BM cells *ex vivo*, we found an increased expression of pDC specific transcription factors E2-2 and SPI1B in *Batf*$^{-/-}$ pDCs, indicating a possible role for BATF in pDC differentiation.
Additionally, results from gene expression profiling of sorted *in vitro* generated WT vs. *Batf*$^{-/-}$ pDCs stimulated with the TLR9 ligand CpG or left untreated will be presented.
In summary, our results hint at a role of BATF in pDC differentiation. Further analyses will focus on unraveling the underlying molecular mechanisms.

P.A1.01.12

Developmental heterogeneity of early life dendritic cells

N. E. Papaioannou[1], *J. Salvermoser*[1,2], *N. Salei*[1,2], *M. Pasztoi*[1], *R. Schuchert*[2,3], *S. E. W. Jacobsen*[4,5], *C. Schulz*[2,3], *B. U. Schraml*[1,2];
[1]*Biomedical Center, LMU Munich, Planegg-Martinsried, Germany,* [2]*Walter-Brendel-Centre for Experimental Medicine, University Hospital, LMU Munich, Planegg-Martinsried, Germany,* [3]*Medizinische Klinik und Poliklinik I, Klinikum der Universität München, LMU, München, Germany,* [4]*Haematopoietic Stem Cell Laboratory, MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom,* [5]*Department of Cell and Molecular Biology, Wallenberg Institute for Regenerative Medicine, Karolinska Institutet, Stockholm, Sweden.*

Dendritic cells (DCs) are immune sentinels that initiate, polarize and orchestrate adaptive and innate immune responses. Early life DCs differ both quantitatively and qualitatively from those in adults. Neonatal DCs, for instance, promote immune responses less efficiently than their adult counterparts, because they trigger reduced T cell proliferation. Additionally, they produce low levels of immunostimulatory cytokines resulting in Th2 biased immune responses. As a consequence, neonates are more susceptible to infection with certain pathogens than adults.
Using a model for fate mapping of conventional DC precursors (CDP) with yellow fluorescent protein (YFP), we have revisited the development of DCs during mouse embryogenesis, as well as in perinatal and adult mice. Surprisingly, we find that $CD11c^+MHCII^+CD11b^+$ splenocytes, which phenotypically resemble CDP-derived $CD11b^+$ DCs that label strongly with YFP in adult mice, were poorly labeled in embryonic and neonatal mice. Combined with additional fate mapping experiments for primitive and definitive hematopoietic progenitors our data suggest that DCs exhibit age-dependent heterogeneity in terms of their ontogeny. Currently, we are investigating the impact of this developmental heterogeneity on the capacity of early life DCs to respond to pathogenic stimuli and initiate immune responses. Taken together, our data suggest that a layered DC development may contribute to the qualitatively altered DC responses in early life.
This work is supported by an ERC Starting Grant awarded to BS (ERC- 2016-STG-715182).

P.A1.01.13

Therapeutic targeting of hexosamine biosynthetic pathway in acute myeloid leukemia

***R. PARAMESWARAN**, A. Asthana;*
CASE WESTERN RESERVE UNIVERSITY, CLEVELAND, United States.

Acute myeloid leukemia (AML) is the most common acute leukemia among adults with an overall poor prognosis. For 40 years there has been minimal improvement in treatment beyond induction chemotherapy or allogeneic stem cell transplantation. Targeting cell metabolism is a promising avenue for future cancer therapy. We found that enzymes involved in metabolic hexosamine biosynthetic pathway (HBP) is increased in AML patients. The HBP metabolizes glucose and glutamine to produce UDP-N-acetylglucosamine (UDP-GlcNAc) which is the substrate for O-GlcNAcylation, a post translational modification on cytosolic and nuclear proteins. We found that glutamine-fructose-6-phosphate-amidotransferase (GFAT), the rate limiting enzyme of HBP pathway and O-GlcNAc transferase (OGT), which catalyzes the addition of GlcNAc to proteins are significantly increased in different AML patient subtypes as well as different AML cell lines. Inhibiting GFAT pushes AML cells to differentiate, causes cell cycle arrest, inhibits cell proliferation and finally leads to apoptotic cell death, sparing normal cells. Knockdown of OGT enzyme using OGT Sh-RNA also led to AML cell differentiation and cell death. Finally, targeting of HBP *in vivo* leads to significant clearance of tumor cells in an AML xenograft mouse model, with minimum toxicity. This study reveals an important role of HBP in keeping AML cells in the un-differentiated and malignant state and sheds light into a new area of potential AML therapy by targeting HBP pathway.

P.A1.01.14

Mesoporous Silicon Microparticles enhance inflammatory responses in human macrophages

***I. Real Arévalo**[1,2], B. Amorós-Pérez[1], A. Revilla[1,2], L. Diego-González[1], B. Martín-Adrados[1], R. Martín-Palma[3], E. Martínez-Naves[1], M. Gómez del Moral[2];*
[1]Departamento de Inmunología. Facultad de Medicina. Universidad Complutense, Madrid, Spain, [2]Departamento de Biología Celular. Facultad de Medicina. Universidad Complutense, Madrid, Spain, [3]Departamento de Física Aplicada, Universidad Autónoma de Madrid, Madrid, Spain.

Introduction: Mesoporous silicon microparticles (MSMPs) possess unique chemical stability, adjustable pore size, extensive surface area, biocompatible and biodegradable nature, that offer large advantages over current adjuvants or vehicles. We have previously described that peptide-loaded MSMPs enhance antigen specific T-cell activation by human monocyte derived dendritic cells. Macrophages are important APCs whose activity can be polarized to a proinflammatory (M1) or anti-inflammatory (M2) phenotype. Aim: Our goal was to investigate how macrophages respond to MSMPs.
Methods: MSMPs were fabricated by electrochemical treatment of silicon wafer. M1 or M2 Macrophages were obtained after 7 days of incubation with GM-CSF or M-CSF respectively, from $CD14^+$ monocytes isolated from Peripheral blood mononuclear cells (PBMCs) using immunomagnetic microbeads. M1 and M2 phenotype was assessed by gene and phenotype expression by quantitative real-time PCR and flow cytometry respectively. Cytokines were quantified in culture supernatants by ELISA. Endocytosis was assesed by flow cytometry using FITC labeled MSMPs
Results: Our results showed that that MSMPs are efficiently endocyted by both M1 and M2 polarized macrophages, in a process that is in part mediated by Scavenger receptors. Enhanced CD80, CD86, HLA-DR and CD163 markers on the cell surface in both M1 and M2 macrophages were observed after MSMP endocytosis. There was an increase of TNFα, IL6 and IL12 secretion in M1 macrophages treated with MSMPs. No production of IL-10 was observed in M1 or M2 cell supernatants.
Conclusions: Microparticles of mesoporous silicon are endocytosed by macrophages favouring a proinflammatory profile (M1) and inhibiting the IL-10 production.

P.A1.01.15

Developmental origin and cellular identity of cardiac macrophages in steady state and in response to stress

***C. Schulz**[1], T. Weinberger[1], V. Schneider[1], E. Gomez Perdiguero[2];*
[1]Ludwig-Maximilians-University, Munich, Germany, [2]Institut Pasteur, Paris, France.

Background: Macrophages are the most prominent immune cells in myocardial tissue and play a critical role in pathological conditions. However, the quantitative contribution of embryonic and bone marrow (BM) hematopoiesis to the cardiac macrophage pool under steady state and in response to stress has been unknown. Methods/Results: In this study we mapped the origin and fate of the different macrophage lineages in mouse models of ischemia-reperfusion injury (I/R) and Angiotensin II-induced fibrosis (AT-II). Using FLT3-Cre mice and radiation-independent BM chimera, we found that under steady state a considerable amount of cardiac macrophages develops independently of definitive hematopoiesis. In response to both I/R and AT-II, we observed an increase in the number of BM-derived macrophages in the heart. However, this increase was only transient and the number of BM-derived macrophages declined over time reaching steady state numbers after 30 days. After AT-II infusion, we found an increase of BM-derived macrophages in areas of myocardial and interstitial fibrosis, whereas after I/R-injury there was a profound influx of BM-derived macrophages in the infarct region and its adjacent remote area. The influx of BM-derived macrophages could be significantly reduced by deletion of CCR2. Conclusion: Embryonic macrophages are the major contributors to the pool of cardiac tissue-resident macrophages in adult mice. In the acute phase of inflammation, BM macrophages invade the heart but do not persist in significant numbers in myocardial tissue. Our findings are of potential relevance for understanding the cardiac immune response and for the therapeutic targeting of macrophages in inflammatory conditions.

P.A1.01.16

TCDD-mediated activation of aryl hydrocarbon receptor alters microRNAs (miRs) expression in Granulocytic Myeloid Derived Suppressor cells (G-MDSCs) and Splenic Resident Granulocytes (SRG) via shared and unique pathways of differentiation

***N. P. Singh**, D. Jackson, U. P. Singh, S. Sumpter, P. Nagarkatti, M. Nagarkatti;*
University of South Carolina School of Medicine, Columbia, United States.

TCDD is an AhR ligand and has potent immunosuppressive effects on humans and animals. Recently, we have shown that TCDD triggers induction of myeloid derived suppressor cells (MDSCs) in peritoneal cavity. In this study, we investigated the effect of TCDD on the regulation of miRs in Granulocytic MDSCs (G-MDSCs) and splenic resident Granulocytes (SRG) in mice. To this end, mice were treated with vehicle or TCDD (10 µg/kg bw) and 48 hours post injection, G-MDSCs from peritoneal wash and SRG from spleen were sorted (>95-96% purity) and microRNA arrays were performed using total RNAs. There was significant alterations in the expression of 78 miRs in both G-MDSCs and SRG. Out of the 78 miRs, 19 miRs were upregulated in G-MDSCs but downregulated in SRG, 16 miRs were downregulated in G-MDSCs but upregulated in SRG, 32 miRs were upregulated in both G-MDSCs and SRG, and the remaining 11 miRs were downregulated in both G-MDSCs and SRG. Upon analysis of TCDD-dysregulated miRs using IPA, more than 25 diseases and pathways are regulated. Thse dysregulated miRs play a common role in the development of several diseases including cancer and inflammatory diseases. This study demonstrate for the first time that TCDD regulates miRs that are both shared and unique to G-MDSCs and SRGs, thereby suggesting that while G-MDSCs are similar in some ways to SRGs, they may be at different stages of differentiation. The present study was supported by NIH grants (R01ES019313, R01MH094755, R01AI123947, R01 AI129788, P01 AT003961, P20 GM103641, R01 AT006888).

P.A1.01.17

Precise delineation and transcriptional characterization of bovine blood dendritic-cell and monocyte subsets

***S. C. Talker**[1,2], A. Baumann[1,2], G. T. Barut[1,2], I. Keller[3], R. Bruggmann[3], A. Summerfield[1,2];*
[1]Institute of Virology and Immunology, Bern and Mittelhäusern, Switzerland, [2]Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [3]Interfaculty Bioinformatics Unit and Swiss Institute of Bioinformatics, University of Bern, Bern, Switzerland.

A clear-cut delineation of bovine bona fide dendritic cells (DC) from monocytes has proved challenging, given the high phenotypic and functional plasticity of these innate immune cells and the marked phenotypic differences between species.
Here, we demonstrate that, based on their expression of Flt3, CD172a, CD13, and CD4, a precise identification of bovine blood conventional DC type 1 and 2 (cDC1, cDC2), plasmacytoid DC (pDC), and monocytes is possible with cDC1 being $Flt3^+CD172a^{dim}CD13^+CD4^-$, cDC2 being $Flt3^+CD172a^+CD13^-CD4^-$, pDC being $Flt3^+CD172a^{dim}CD13^-CD4^+$, and monocytes being $Flt3^-CD172a^{high}CD13^-CD4^-$. The phenotype of these subsets was characterized in further detail, and a subset-specific differential expression of CD2, CD5, CD11b, CD11c, CD14, CD16, CD40, CD71, CD163, CD205, and CADM1 was found. Subset identity was confirmed by transcriptomic analysis and subset-specific expression of conserved key genes. We also sorted monocyte subsets based on their differential expression of CD14 and CD16. Classical monocytes ($CD14^+CD16^-$) clustered clearly apart from the two $CD16^+$ monocyte subsets probably representing intermediate and non-classical monocytes described in human. The transcriptomic data also revealed differential expression of molecules involved in antigen presentation, pathogen sensing, and migration, and therefore gives insights into functional differences between bovine DC and monocyte subsets.
The identification of cell-type- and subset-specific gene expression will assist in the quest for “marker molecules” that – when targeted by flow cytometry – will greatly facilitate research on bovine DC and monocytes. Overall, species comparisons will elucidate basic principles of DC and monocyte biology and will finally help to translate experimental findings from one species to another.

P.A1.01.18

Th1 expansion is restrained by IFN-γ-induced adherence-independent macrophages and restored by PD-L2 blockade

***E. Tavukcuoglu**, G. Esendagli;*
Hacettepe University Cancer Institute Department of Basic Oncology, Ankara, Turkey.

Monocyte-to-macrophage differentiation is simultaneously induced ex-vivo and regulated according to the cytokine milieu and the matrix adherence. This study originates from a random laboratory observation while testing stimulatory potential of macrophages differentiated in suspension cultures. Under defined conditions lacking activation stimuli, exposure to a low concentration of interferon (IFN)-γ gave rise to macrophages with impaired T-cell stimulation capacity. B7 family of immune regulatory ligands, CD80, CD86, ICOS-LG, B7-H3, and especially PD-L1 and PD-L2, were modulated on the IFN-γ-induced adherence-independent monocyte-derived macrophages. These cells supported helper T-cell (Th) activation but had limited capacity to stimulate Th1 differentiation and proliferation when compared to the control macrophages. PD-L2, which has been identified with contradictory effects on T-cell responses, was highly present on both control and IFN-γ-induced cells. The blockade of this PD-1 ligand significantly accelerated Th1 proliferation and restored the secretion of effector cytokines, especially IL-2 and IFN-γ. Collectively, the high and constitutive expression of PD-L2 on macrophages did not impede the establishment of functional capacity in Th-cells but interfered with Th1 proliferation and cytokine production. Therefore, our data indicate the role of macrophage PD-L2 as a safety measure that reins acceleration of Th1 responses.This project was supported by Hacettepe University Research Fund (Project no. TSA-2017-12739).

P.A1.01.20

Role of serum Hypoxia-Inducible Factor (HIF)-1α in patients with COPD

***k. upadhyay**, a. ali, p. sohal, a. yadav, s. p. bhatt, r. guleria;*
all india institute of medical sciences, new delhi, India.

Introduction: - HIF-1α protein is a master transcriptional regulator of the adaptive response to hypoxia which increases with initiation of oxidative stress. However, the significance of this protein in COPD patients with smoking habit is still unknown.
Aim: – The study aims to evaluate the role of HIF-1α in smoker COPD patients with an emphasis to its association with disease severity.
Method: - In this case controlled study, we enrolled 57 COPD patients, along with 15 smokers without COPD and 15 healthy individuals (as control sets). The mean age of patients was 54.6 (± 9.32) years and controls 50.0 (± 9.8). The study set included 62% smokers, 25% non-smokers, 7% tobacco chewers and 6% ex-smokers. Sandwich Enzyme-linked immune Sorbent Assay (ELISA) method was used for analyzing serum HIF-1α.
Results: - A significantly higher HIF-1α level was observed in COPD patients with smoking habit, indicating a positive association with hypoxia, smoking status and severity of disease (p=0.03). However, the mean value of HIF-1α was not significantly different in smokers without COPD and healthy controls.
Conclusion: - Our study indicates positive association of HIF-1α levels with smoking habit of COPD patient and severity of the disease unlike the smokers without COPD suggesting that activation of HIF-1α pathway in COPD.

P.A1.01.21

Evolutionary conservation of bone marrow-derived antigen presenting cells

***R. van den Biggelaar**[1], V. Rutten[1,2], W. van Eden[1], C. Jansen[1];*
[1]Utrecht University, Utrecht, Netherlands, [2]Pretoria University, Pretoria, South Africa.

Dendritic cells (DCs) are antigen presenting cells (APCs) that bridge the innate and the adaptive immune system. Many studies on DCs are based on *in vitro* differentiation of human monocyte- or murine bone marrow-derived progenitor cells into APCs in the presence of GM-CSF and IL-4. Whether these cells resemble their *in vivo* counterpart is still under debate. Recently, culture methods have been developed to generate chicken bone marrow-derived APCs. We aim to characterize this heterogenic cell population in chickens and to study "evolutionary conservation" of APCs in this non-mammalian species. Cells isolated from femurs and tibiae of chicken embryos were cultured in the presence of recombinant chicken IL-4 and GM-CSF. At day 7, these immature APCs were matured by LPS stimulation. Flow cytometry, fluorescent microscopy, and RT-qPCR were performed to characterize these cells. The majority of the cells are CD11b/c^{+}CD206 (MRC1)$^{+}$CD117 (cKit)$^{+}$CD40^{+}. Within this population, half of the cells are MHCII^{high}CD80^{high}CD115 (CSF1R)^{low} and resemble mammalian DCs. The other cells are MHCII^{low}CD80^{low}CD115 (CSF1R)^{high} and resemble macrophage-like cells. The latter are larger in size and have a higher phagocytosis capacity than MHCII^{high} cells. Similar subpopulations have been observed in murine bone marrow-derived DCs. However, murine macrophage-like MHCII^{low} cells are known to lack expression of CD40 or cKit, in contrast to the MHCII^{low} subpopulation that we have observed. Despite these differences, murine and avian bone marrow-derived APCs show high similarity, suggesting an evolutionary conservation of APC marker expression and potentially their role within the immune response in mammalian and non-mammalian species.

P.A1.01.22

TIPE2 Protein Specifies the Functional Polarization of Myeloid-derived Suppressor Cells during Tumorigenesis

***D. Yan**[1], J. Wang[1,2], M. Xu[1,2], O. Adeleye[1], Y. Chen[3], X. Wan[1];*
[1]Chinese Academy of Sciences, Shenzhen, China, [2]Jinan University, Guangzhou, China, [3]University of Pennsylvania, Philadelphia, United States.

Myeloid-derived suppressor cells (MDSCs) are "polarized" myeloid cells that effectively promote tumorigenesis by inhibiting anti-tumor immunity. How myeloid cells acquire the pro-tumoral properties during tumorigenesis is poorly understood. We report here that the polarity protein TIPE2 (tumor necrosis factor-α-induced protein 8-like 2) mediates the functional polarization of MDSCs by specifying their pro- and anti-tumoral properties. Tumor cells induced the expression of TIPE2 in Gr1^{+}CD11b^{+} cells through reactive oxygen species (ROS). TIPE2 in turn increased the expression of pro-tumoral mediators such as CCAAT/enhancer-binding protein-β while inhibited the expression of anti-tumoral mediators. Consequently, tumor growth in TIPE2-deficient mice was significantly diminished, and TIPE2-deficient MDSCs markedly inhibited tumor growth upon adoptive transfer. Pharmaceutical blockade of ROS inhibited TIPE2 expression in MDSCs and reduced tumor growth in mice. These findings indicate that TIPE2 plays a key role in the functional polarization of MDSCs and represents a new therapeutic target for cancer immunotherapy.
This work was supported by the Natural Science Foundation of China (Grant 81501356 to D.Y. and Grant 81373112 to X.W.), the Shenzhen Basic Science Research Project (Grants JCYJ20160229201353324 to D.Y., JCYJ20170413153158716 to X.W.).

P.A1.01.23

Identification and characterisation of XCR1+ avian conventional dendritic cells

*Z. Wu[1], M. McGrew[1], H. Sang[1], D. Hume[2], **A. Balic**[1];*
[1]The Roslin Institute, Edinburgh, United Kingdom, [2]University of Queensland, Brisbane, Australia.

Comparative transcriptomic studies have enabled the identification of conventional dendritic cells (cDC) in a large number of different mammalian species and more recently chickens (J Immunol. 2014. 192(10):4510-7). While these studies elegantly demonstrate the evolutionary conservation of cDC in aminotes, little information is available on the specific developmental and functional biology of avian cDC. This is largely due to the lack of specific reagents and immunological tools to identify these cells in the chicken. We have developed specific reagents that in combination with the CSF1R-eGFP transgenic reporter chicken allow the specific identification, characterisation and isolation of chicken cDC. We will show that expression of Flt3 and the chemokine XCR1 enables the discrimination between chicken cDC and macrophages as well as isolation for functional studies. In mammals cDCs can be classified into two functionally distinct lineages, in contrast we are can identify only a single cDC subset in chickens. While this chicken cDC subset closely resembles the mammalian cDC1 subset, substantial differences also also observed, likely reflecting the unique biology of the avian immune system. Furthermore while we readily detect cDC in avian tissues, in contrast to mammals we are unable to detect either circulating cDC nor cDC progenitors in chickens. In this presentation we will discuss the specific developmental and functional biology of avian cDC and contrast this to what is known of mammalian cDC, as well as present an update in on the progress making a cDC knockout line of chickens. In particular we will discuss the implications of only having a single identifiable cDC lineage in chickens.

P.A1.01.24

Distinct progenitor lineages contribute to the heterogeneity of plasmacytoid dendritic cells

*P. F. Rodrigues[1], L. Alberti-Servera[1,2], A. Eremin[1], G. E. Grajales-Reyes[3], R. Ivanek[1,4], **R. Tussiwand**[1];*
[1]Department of Biomedicine University of Basel, Basel, Switzerland, [2]Department of Human Genetics and VIB Center for the Biology of Disease, Leuven, Belgium, [3]Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, United States, [4]Swiss Institute of Bioinformatics, Basel, Switzerland.

Plasmacytoid dendritic cells (pDCs) are an immune subset devoted to the production of high amounts of type 1 interferons in response to viral infections. Whereas conventional dendritic cells (cDCs) originate mostly from a common dendritic cell progenitor (CDP), pDCs have been shown to develop from both CDPs and common lymphoid progenitors. Here, we found that pDCs developed predominantly from IL-7R^{+} lymphoid progenitor cells. Expression of SiglecH and Ly6D defined pDC lineage commitment along the lymphoid branch. Transcriptional characterization of SiglecH^{+}Ly6D^{+} precursors indicated that pDC development requires high expression of the transcription factor IRF8, whereas pDC identity relies on TCF4. RNA sequencing of IL-7R^{+} lymphoid and CDP-derived pDCs mirrored the heterogeneity of mature pDCs observed in single-cell analysis. Both mature pDC subsets are able to secrete type 1 interferons, but only myeloid-derived pDCs share with cDCs their ability to process and present antigen.

P.A1.02 Myeloid lineage specification - Part 2

P.A1.02.01
Mitochondrial dynamics in macrophage biology

A. H. R. Agnellini[1], *R. Sanchez*[1], *C. Tezze*[1], *G. I. Toffolo*[1], *M. Sandri*[1], *B. Molon*[2], *L. Scorrano*[3], *A. Viola*[1];
[1]*Department of Biomedical Science, University of Padova, Padova, Italy, Padova, Italy,* [2]*Barbara Molon, Padova, Italy,* [3]*Department of cellular Biology, Università degli studi di Paova, Padova, Italy.*

Recent evidence highlights that mitochondrial dynamics unroll specialized functions in the immune system, regulating T cell metabolism and fate. However, the effective relevance of mitochondria in myeloid cell biology remains to be elucidated. Among myeloid cells, macrophages represent a key component of the innate immune system, playing critical roles in host defence and homeostasis. In order to investigate the functional significance of mitochondrial dynamics in macrophages, we generated a tissue-specific conditional knock out mouse model, presenting the selective deletion of optic atrophy 1 (OPA-1) in lysozyme M expressing cells. OPA1-/- mice show a significant reduction of all myeloid populations in all tissues analysed (i.e. blood, spleen, liver and bone marrow). *In vitro*, the absence of OPA1 in macrophages promotes migration, phagocytosis and reactive oxygen species production, but impairs M1/M2 polarization. In order to understand the impact of OPA1 in macrophages polarization in vivo, we analyzed macrophages during muscle regeneration after cardiotoxin injury. Interestingly, we observed slow regeneration in OPA1-/- mice followed by macrophages accumulation. Our project aims to understand how OPA1 deletion orchestrate macrophages metabolism and polarization.

P.A1.02.02
Murine scavenger receptor CD163 is a marker for a specific IRF8-dependent resident macrophage subpopulation in the bone marrow

L. Fischer-Riepe[1], *J. Schulte-Schrepping*[2], *M. Pohlen*[3], *J. Fischer*[4], *T. Ulas*[2], *C. Schulz*[5], *S. Vettorazzi*[6], *F. Rosenbauer*[4], *J. Tuckermann*[6], *J. Schultze*[2], *J. Roth*[1], ***K. Barczyk-Kahlert***[1];
[1]*Institute of Immunology, University of Muenster, Muenster, Germany,* [2]*Genomics and Immunoregulation, Life and Medical Sciences Institute, University of Bonn, Bonn, Germany,* [3]*Institute of Immunology, University of Muenster; Department of Medicine A, Hematology and Oncology, University Hospital of Muenster, Muenster, Germany,* [4]*Institute of Molecular Tumor Biology, University of Muenster, Muenster, Germany,* [5]*Medizinische Klinik und Poliklinik I, Klinikum der Universität München, Ludwig-Maximilians-Universität, Munich, Germany,* [6]*Institute of Comparative Molecular Endocrinology (CME), University of Ulm, Ulm, Germany.*

CD163 is a scavenger receptor exclusively expressed on monocytes/macrophages. Anti-inflammatory factors like glucocorticoids (GC) and IL-10 increase the CD163 expression. CD163 has been excessively studied in man and rat, nevertheless very little is known regarding the structure and regulation of CD163 expression in mice. Here we discovered a $CD163^+$ macrophage population in the murine bone marrow which descends from embryonic progenitors in the yolk sac and is dependent on the transcription factor IRF8.
We could show, that freshly isolated bone marrow cells (BMC) have shown high expression of CD163, which decreased during *in vitro* culture. Stimulation of BMCs with GC restored CD163 expression. This effect was not that pronounced in bone marrow-derived macrophages (BMDM). We identified two monocyte/macrophage subsets in murine BM defined by the expression of CD163. $CD163^+$ BMC were phenotypically and functionally distinct from $CD163^-$ BMC. This specific $CD163^+$ population was missing in the bone marrow and spleen of IRF8-/- mice but not in the other organs tested. Moreover, *in vitro* treatment with GC failed to induce CD163 expression in BMC isolated from $IRF8^{-/-}$ mice. Cell fate studies in Csf1rMer-iCre-Mer;RosaLSL-GFP mice revealed yolk sac origin of $CD163^+$ macrophages. We could observe a strong infiltration of $CD163^+$ cells in allergic, bacterial and parasite disease models. Moreover, the adoptive cell transfer of $CD163^+$ BMC ameliorated the signs of disease in local *S. aureus* infection.
Thus, CD163 expression in murine BM (and spleen) is restricted to a small subpopulation of monocytes/macrophages which is originally, phenotypically and functionally distinct from $CD163^-$ monocytes/macrophages.

P.A1.02.03
Human primary macrophages show tissue-specific profiles of IgG receptor expression

C. W. Bruggeman[1], *S. Q. Nagelkerke*[1], *E. Mul*[2], *M. Hoogenboezem*[2], *J. Houtzager*[3], *B. Dierdorp*[4], *J. Kers*[5], *S. Pals*[5], *R. Lutter*[4], *T. van Gulik*[3], *J. M. den Haan*[6], *T. K. van den Berg*[1], *R. van Bruggen*[1], *T. W. Kuijpers*[1,7];
[1]*Department of Blood Cell Research, Sanquin Research and Landsteiner Laboratory, Academic Medical Center (AMC), University of Amsterdam, Amsterdam, Netherlands,* [2]*Department of Central Facility Research, Sanquin Research and Landsteiner Laboratory, AMC, University of Amsterdam, Amsterdam, Netherlands,* [3]*Department of Experimental Surgery, AMC, University of Amsterdam, Amsterdam, Netherlands,* [4]*Department of Experimental Immunology, AMC, University of Amsterdam, Amsterdam, Netherlands,* [5]*Department of Pathology, AMC, University of Amsterdam, Amsterdam, Netherlands,* [6]*Department of Molecular Cell Biology and Immunology, VU University Medical Center, Amsterdam, Netherlands,* [7]*Emma Children's Hospital, AMC, University of Amsterdam, Amsterdam, Netherlands.*

Tissue-resident macrophages play an important role in the clearance of IgG-opsonized particles and immune complexes via the interaction with IgG receptors, the so-called Fc-gamma receptors (FcγRs). To date most studies investigating the phagocytosis of opsonized particles made use of *in vitro* cultured monocyte-derived macrophages. For comparison, we investigated the FcγR expression on tissue macrophages, both stained in tissue sections and *ex vivo* when freshly purified from those same human tissues. Upon isolation of primary human macrophages from bone marrow, spleen, liver and lung, we observed that macrophages from all studied tissues expressed high levels of FcγRIIIa, which was in direct contrast with blood monocyte-derived macrophages. Moreover, expression levels of FcγRI were highly variable. Kupffer cells in the liver were the only tissue-resident macrophages that expressed the inhibitory IgG receptor FcγRIIb, which is likely to have an important role by their anatomical position in filtering the blood from the portal vein without excessive pro-inflammatory reactivity. Functional experiments with isolated splenic red pulp macrophages demonstrated the contribution of FcγRs in phagocytosis. In sum, our immunohistochemistry data combined with *ex vivo* immunostainings and functional assays of isolated human tissue macrophages indicated that tissue-resident macrophages are different from monocyte-derived macrophages showing distinct tissue-specific FcγR expression patterns.

P.A1.02.04
Distribution of different subsets of monocytic/macrophage cells in human lymphoid tissues through life

D. Damasceno[1], *J. Almeida*[1], *C. Teodosio*[2], *W. B. van den Bossche*[3], *M. Perez-Andres*[1], *S. de Arriba*[4], *A. Romero*[5], *N. Puig*[6], *L. Muñoz-Bellvis*[7], *J. Vicente-Villardón*[8], *J. J. van Dongen*[2], *A. Orfao*[1];
[1]*Cancer Research Center (IBMCC, USAL-CSIC), Cytometry Service (NUCLEUS), Salamanca, Spain,* [2]*Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands,* [3]*Department of Immunology, Erasmus University Medical Center, Rotterdam, Netherlands,* [4]*Service of Pediatrics, University Hospital of Salamanca (Complejo Asistencial Universitario de Salamanca, CAUSA), Salamanca, Spain,* [5]*Health Center "Miguel Armijo". Primary Health Care of Salamanca, Sanidad de Castilla y León (SACYL), Salamanca, Spain,* [6]*Service of Hematology, University Hospital of Salamanca (CAUSA) and IBSAL, Salamanca, Spain,* [7]*Service of Surgery, University Hospital of Salamanca (CAUSA), Department of Surgery of the University of Salamanca and IBSAL, Salamanca, Spain,* [8]*Statistics Department, University of Salamanca, Salamanca, Spain.*

Introduction: Normal human peripheral blood monocytes include the so-called classical ($CD14^{hi}CD16^-$), intermediate ($CD14^{hi}CD16^+$) and non-classical ($CD14^{-/lo}CD16^+$) monocytes. Despite the presence of these monocytic cell compartments has previously been reported in healthy subjects, data about their kinetics through life as well as their relative distribution in secondary lymphoid tissues, remains very limited. Material and methods: Here we investigated the distribution of the distinct subsets of monocytic cells in 11 cord blood and 177 peripheral blood healthy samples through life and their relative distribution in 9 bone marrow, and 11 lymph node and spleen samples. Results: Overall, we identified five distinct subsets of mature monocytic cells in peripheral blood, bone marrow, lymph node and spleen. In cord blood and individuals ≥50y up to 92y ($p<0.05$), higher numbers of classical monocytes were found, while the highest number of intermediate and non-classical monocytes was observed few days after birth ($p<0.05$). In addition, a decreased frequency of circulating $CD62L^+$ classical monocytes was detected in both lymph nodes and spleen tissues vs peripheral blood ($p<0.01$). Conclusions: Altogether, these results support the notion that classical monocytes are recruited to different tissues, where greater numbers of intermediate and non-classical monocytes are generated; in turn, these later two cell populations might recirculate into the bloodstream through the lymph system. This study provides also novel information on age-related kinetics of circulating monocytic subsets, to be used as a frame of reference for normal values. Grants: CB16/12/00400 (CIBER-ONC) and PI13/01412, ISCIII (Ministerio de Economía y Competitividad, Madrid, Spain and FONDOS FEDER).

P.A1.02.05
Signalling networks governing hematopoietic stem cells differentiation during inflammation and pathology

M. De Zuani, *M. Hortová Kohoutková, S. S. Jose, J. Frič;*
International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic.

The differentiation of hematopoietic stem and progenitor cells (HSPCs) to the myeloid and lymphoid lineages is a tightly regulated process that occurs almost exclusively in the bone marrow. Several signals coming from the host as well as from microbial components are able to skew the differentiation of HSPCs toward different cell fates, also acting directly on HSPCs receptors. Similarly, pathological condition (as infections or cancer) and the physiological process of aging are correlated with an altered hematopoiesis which promotes myelopoiesis over lymphopoiesis. A growing body of evidence suggests that HSPCs respond directly to microbial ligands signalling trough TLRs. This process is particularly relevant in the light of the fact that HSPCs are able to recirculate from the bone marrow to the blood stream, thus getting exposed to several PAMPs. However, the dynamics and involvement of TLRs and CLRs signalling during HSCs differentiation have been only poorly investigated.

Taking advantage of flow cytometric techniques and co-culture with 3D organoids, we show that PRRs ligation by microbial products is able to drive human HSPCs differentiation. Thus, we aim to unravel the molecular mechanisms underlying this process by analysing the role of downstream signalling and the activation of specific genetic programs in a human model of hematopoiesis.
The research is supported by European Social Fund and European Regional Development Fund—Project MAGNET (No. CZ.02.1.01/0.0/0.0/15_003/0000492)

P.A1.02.06
Altered immune responses in mice with LST1 adaptor protein deficiency

M. Fabisik[1], *J. Králová*[1], *J. Tureckova*[2], *S. Borna*[1], *T. Skopcova*[1], *J. Prochazka*[2], *F. Spoutil*[2], *B. Malissen*[3], *R. Sedlacek*[2], *T. Brdicka*[1];
[1]*Institute of Molecular Genetics of the ASCR, v. v. i., Prague, Czech Republic,* [2]*Czech Centre for Phenogenomics, hosted by the Institute of Molecular Genetics ASCR, Prague, Czech Republic,* [3]*Centre d'Immunologie de Marseille Luminy, Aix Marseille Université, INSERM, CNRS UMR, Marseille, France.*

Transmembrane adaptor protein LST1 is expressed in leukocytes of the myeloid lineage. Previous study has revealed mild effects of LST1 deficiency on the outcome of influenza infection in mice. Except for this specific case, its overall function at the organismal level is still to be determined. At the molecular level, LST1 was shown to interact with cytoskeleton regulating proteins and to promote the formation of tunneling nanotubes. It also contains an ITIM motif in its intracellular tail, which was shown to bind phosphatases SHP-1/2 in monocytes. To study the physiological function of LST1 we have performed a thorough analysis of LST1-deficient mice. At steady state, these mice displayed no apparent phenotype. However, when we challenged LST1-deficient mice with pro-inflammatory stimuli some aspects of their responses were altered. Intraperitoneal injection of viral mimetic PolyI:C resulted in significant reduction in splenic $CD8^+$ T cell percentages. However, the most striking differences were observed when we induced acute colitis in these mice by dextran sodium sulphate, as a model of disease, where myeloid cells are heavily involved. We found significantly better course of acute colitis in LST1-deficient animals in all observed parameters. This was accompanied by alterations in splenic monocyte populations. Interestingly, we also saw the same significant decrease in CD8+ splenic T cells as after polyI:C injection. Collectively our data suggest, that LST1 is not required for leukocyte development and immune system homeostasis, but it is involved in the regulation of several types of immune responses. Financed by GACR P302/12/G101.

P.A1.02.07
Experimental human endotoxemia results in accelerated maturation of the neutrophil compartment in the human bone marrow

E. van Grinsven[1,2], *S. van Staveren*[1,2], ***M. Hassani***[1,2], *K. Tesselaar*[1], *G. Leijte*[3], *M. Kox*[3], *P. Pickkers*[3], *N. Vrisekoop*[1,2], *L. Koenderman*[1,2];
[1]*Laboratory of translational immunology, Utrecht, Netherlands,* [2]*University Medical Center Utrecht, Utrecht, Netherlands,* [3]*Radboud University Medical Center, Nijmegen, Netherlands.*

Neutrophils are thought to be short-lived cells and are produced in extremely high numbers in the bone marrow (>10^{11} cells/day). Nevertheless, solid data to support this hypothesis is lacking. We investigated the proliferation and maturation of the neutrophil compartment in humans during homeostasis and experimental human endotoxemia, as *in vivo* model for acute systemic inflammation .
Bone marrow and blood were obtained from healthy volunteers before and during acute systemic inflammation evoked by intravenous challenge with endotoxin (lipopolysaccharide, 2 ng/kg) . By differential CD16/CD11b staining promyelocytes, myelocytes, metamyelocytes, banded cells and mature cells were identified and sorted for analysis on cytospins. Differentiation was also studied with a phenotyping panel, containing Ab against CD305, CD49d, , CD16, CD62L, CD11b, CD35, CD66b, CD13, CD11c and CD10. Four hours after endotoxin-challenge, the neutrophil compartment exhibited signs of accelerated maturation, characterized by an increased number of cells with more mature characteristics in all neutrophil fractions. Although large numbers of banded neutrophils were found in the peripheral blood, these cells in the bone marrow did not decrease. Also, the number of $CD62L^{low}$ neutrophils increased in both blood and bone marrow.
In conclusion, our study shows that the neutrophil compartment in the bone marrow of healthy volunteers responds immediately to systemic inflammatory cues and shows signs of accelerated maturation. During short term inflammation, the release of neutrophils with banded or hypersegmented nuclei does not lead to depletion of these cells in the bone marrow.

P.A1.02.08
Multiparametric *in situ* characterisation of macrophages in gastric cancer

Y. Huang[1,2], *M. Wang*[1,2], *Y. Sun*[2], *N. Di Costanzo*[1,2], *C. Mitchell*[3], *A. Achuthan*[4], *R. Busuttil*[1,2], *J. Hamilton*[4], *A. Boussioutas*[1,2,4];
[1]*Upper Gastrointestinal Translational Research Laboratory, Peter MacCallum Cancer Centre, Melbourne, Australia,* [2]*Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, Australia,* [3]*Department of Pathology, Peter MacCallum Cancer Centre, Melbourne, Australia,* [4]*Department of Medicine, The University of Melbourne, Royal Melbourne Hospital, Melbourne, Australia.*

Introduction: Tumour-associated macrophages (TAMs) are one of the most important components of the microenvironment in gastric cancer (GC) and have been shown to exhibit immune suppressive and tissue remodelling properties. The M1/M2 system is commonly used to classify TAMs but may represent an oversimplification of a continuum of macrophage phenotypes. Despite intense characterisation of *in vitro* polarised macrophages, their heterogeneity *in situ* is not well understood. We performed a comprehensive study to identify and characterise the macrophage populations in the GC microenvironment and to determine their relevance to clinical outcome. **Materials and Methods:** A macrophage specific seven-colour immunohistochemistry panel was applied to 56 formalin-fixed paraffin-embedded GC patient specimens. Multiple images of each specimen were analysed to account for tumour heterogeneity. Samples were phenotyped using the inForm software (PerkinElmer). Cell number and spatial distribution were analysed with R. **Results:** Seven unique TAM subtypes were identified. The predominant subtype located at the tumour margin (interface of tumour and adjacent tissue) consisted of CD68+CD163+CD206+ cells while the CD68+IRF8+ population was significantly increased within the tumour core. However, the presence of neither population was correlated with patient outcome. The CD163+ (CD206-) TAMs were associated with improved survival in patients where these macrophages were both abundant in the tumour core and in direct contact (10µm) with the tumour cells. **Conclusions:** Macrophages are heterogeneous even within the same tissue. Spatially resolving their density and co-localisation with the tumour cells could help understand their interactions, provide better survival predictions and identify possible therapeutic candidates for GC patients.

P.A1.02.09
CD11b Expressionin CGD patients and its use in X-CGD carrier detection

M. Y. Koker, *B. Saraymen, E. Bentli;*
Erciyes medical school, Kayseri, Turkey.

Chronic granulomatous disease (CGD) is a rare primary immunodeficiency disorder of phagocytes resulting in impaired killing of bacteria and fungi. X-linked CGD have been reported to be responsible for approximately 65% of all CGD cases and AR-CGD is 35% of them.
The CD11b antigen is known as the integrin αM subunit. CD11b is highly expressed on neutrophils, monocytes and macrophages. Oxidative burst activity and the expression of CD11b_CD18 have been used as indicators of leukocyte activation status. CD11b_CD18 integrins on the PMN membrane not only mediate cell adhesion, but they also marker for activation of protein tyrosine kinases.Neutrophil function were controlled with DHR assay in 24 samples 8 controls, 8 X-CGD patient, 8 X-CGD Carrier. Than, this assay were done with CD11b antibody and PMA by using same blood samples without dihydrorhodamine 123 . For this purpose 100 microL fresh peripheral blood sample is used for standart flow cytometric procedure. After stimulation of (PMA), the expression CD11b on neutrophil was measured by flow cytometric analysisThe results were compared with DHR 123 assay. There was a highly significant correlation between DHR assay and CD11b with stimulation analysis in flow cytometry application in all this samples. Especially CD11b with stimulation testing in the X-CGD Carrier mothers of neutrophil percentages exactly the same from both DHR 123 assay. Beside DHR assay, specific antibody for CD11b could also be used for functional evaluation of neutrophil. It is useful for alternative parameter for diagnosis and carrier screening of X-CGD patients like in DHR-123 assay.

P.A1.02.10
Ontogeny of tissue-resident macrophages during blood-stage malaria

S. Lai[1,2], *C. Ruedl*[1];
[1]*School of Biological Sciences, Nanyang Technological University, Singapore, Singapore,* [2]*Singapore Immunology Network, A*STAR, Singapore, Singapore.*

Blood-stage malaria potently initiates both innate and adaptive immune responses, inclusive of the activation and modulation of the mononuclear phagocyte network. *Plasmodium* infection results in a profound loss of embryonically established tissue-resident macrophages primarily in the spleen, heavily involving the liver and lungs; and whose numbers are restored following clearance of *Plasmodium* parasites. During acute blood-stage malaria, both self-renewing tissue-resident macrophages and blood monocytes contribute to the repopulation of the emptied niches of splenic red-pulp macrophages and liver Kupffer cells, unlike lung alveolar macrophages that are capable of refilling their niche mainly through self-renewal. Despite *Plasmodium* infection, the local tissue microenvironment of spleen and liver allows the replenished macrophages to gain almost identical gene expression profiles and turn-over kinetics as the original foetal-derived counterparts. Thus, the mononuclear phagocytic system has developed distinct, but effective tissue-dependent strategies to efficiently refill available niches, which were rendered accessible during the *Plasmodium* infection.

P.A1.02.11

Myeloid-Derived Suppressor Cells originate from bone marrow are induced by G-CSF and persist in septic shock

E. Lereclus[1,2]*, T. Daix*[3]*, E. Guérin*[4,1]*, B. François*[3,2]*, J. Feuillard*[1,4]*, S. Alain*[2]*, R. Jeannet*[1]*;*
[1]UMR CNRS 7276, Medicine University, Limoges, France, [2]UMR Inserm 1092, Medicine University, Limoges, France, [3]Medical-surgical ICU, Dupuytren Teaching Hospital, Limoges, France, [4]Hematology Laboratory, Dupuytren Teaching Hospital, Limoges, France.

Introduction: Myeloid-derived suppressor cells (MDSC) are composed by a polymorphonuclear (PMN-MDSC) and a monocytic (M-MDSC) subset. Their functions are well described in cancer but it remains unclear how they are produced and recruited during sepsis. **Methods:** 57 immunocompetent patients hospitalized in Limoges Hospital for an acute infection were enrolled and regrouped according to their clinical severity: infection, sepsis and septic shock. We followed the level of M-MDSC and PMN-MDSC by flow cytometry in Peripheral Blood (PB) at D0, D3, D7 and D14. Plasma was collected to perform pro-inflammatory cytokines analysis. 20 residual Bone Marrow (BM) samples for thrombocytopenia diagnosis at admission were availabled for MDSC analysis. BM without hematological diseases were used for cultured assay. **Results:** When compared to healthy controls at admission, all patients showed an increase of MDSC absolute number in PB regardless of severity. Similarly, cytokine storm is visible in all patients, but cytokine decrease is slower in the most severe patients. MDSC were functional *in-vitro* and normal BM were able to produce M-MDSC and PMN-MDSC in presence of G-CSF and GM-CSF. Finally, MDSC are present in the BM of septic and septic shock patients with thrombocytopenia. **Conclusion:** We demonstrated that MDSC are produced by the BM following an infection and released rapidly in the PB. Interestingly, a sustain or increase number of MDSC after 3 days of hospitalization is linked to the severity of the sepsis and cytokine level in PB. Survival analyses are under investigation.

P.A1.02.12

Identification of monocytes from Asian elephant (*Elephas maximus*) by flow cytometry confirms a large population of PBMC with a diverse morphological profile

T. Maehr[1,2]*, A. Dastjerdi*[2]*, J. Lopez*[3]*, F. Steinbach*[1,2]*;*
[1]School of Veterinary Medicine, Guildford, United Kingdom, [2]Animal and Plant Health Agency (APHA), Weybridge, United Kingdom, [3]Chester Zoo, Upton by Chester, United Kingdom.

Asian elephants (*Elephas maximus*) are a critically endangered species where captive and wild populations threatened by a range of pathogens such as Elephant endotheliotropic herpesviruses (EEHV). EEHV can cause a fatal haemorrhagic disease (HD) in juveniles and the inability to culture the virus in combination with a lack of understanding of the elephant's immune system has hampered the development of efficient prophylactic. Specifically, only a few reagents are available for the analysis of the elephants' immunity, which may play a critical role in containing herpesvirus infections.
We assessed a panel of commercially available monoclonal antibodies (mAb's) for cross-reactivity and suitability to characterise elephant subsets. Initial flow cytometric analysis of peripheral blood mononuclear cells (PBMCs) revealed a forward and side scatter (FSC/SSC) pattern that is different when compared to other species, indicating a large putative monocyte population (>30%). This was corroborated by the staining pattern of an anti-bovine CD14 mAb found to have inter-species reactivity with elephant PBMCs whereby $CD14^{+}$ cells corresponded to two potential monocyte subsets in FSC/SSC plots. In addition, an anti-MHCII, an anti-CD3 and a mAb against B cells react with elephant leukocytes. Together these Ab's indicate an unusual composition of immune cells in Asian elephant that requires further investigation into the correlation with age and to better understand how monocytes dominate the PBMCs.

P.A1.02.13

Functional alterations in monocytes derived from bone marrow of patients with myelodysplastic syndrome

L. B. Cameirão[1]*, N. S. Bacal*[2]*,* ***L. Marti***[3]*;*
[1]Hospital Israelita Albert Einstein - Clinical Pathology Laboratory, São Paulo, Brazil, [2]Centro de Hematologia de São Paulo and Hospital Israelita Albert Einstein, São Paulo, Brazil, [3]Hospital Israelita Albert Einstein - Instituto Israelita de Ensino e Pesquisa Albert Einsten, São Paulo, Brazil.

Background Myelodysplastic syndrome (MDS) comprises heterogeneous group of clonal hematopoietic stem cell diseases with variable clinical course and risk of evolution to acute myeloid leukemia. Immunophenotypic changes of monocytic lineage are often seen in MDS. **Aims**: To evaluate functionality of monocytes with abnormal CD56 expression isolated from bone marrow of MDS patients. **Methods:** All samples were obtained after patients or controls signed an informed consent approved under CAAE number 33739214.8.0000.0071. All patient samples (05) and controls (03) were evaluated for presence of anomalous CD56 expression and for monocytes subsets percentage using flow cytometry. Monocytes with abnormal CD56 expression were isolated using anti-CD56/CD14 magnetic beads. The isolated cells were cultured with GM-CSF and IL-4 in order to verify their ability of differentiation into dendritic cells . Monocytes phagocytic potential was also evaluated using phagocytosis assay with fluorescent *E. coli* bioparticles. **Results:** All patient samples presented phenotypic anomalies, and significant decrease in intermediate monocyte subpopulation compared to controls (p=0.0082). Dendritic cells derived from CD56 monocytes showed important morphological alterations in size and decreased/absence of dendrites formation. We also observed immunophenotypic changes in expression of molecules such as decreased HLA-DR and CD209 compared to controls (p=0.0035; p=0.049) respectively. Moreover, there was a significant decrease in phagocytic capacity in monocytes from MDS patients (p<0.001). **Conclusion:** Dendritic cells derived from MDS patients with or without aberrant expression of CD56 showed morphological and immunophenotypic alterations. Monocytes also displayed reduced phagocytic capacity. These changes are important and should be investigated in the context of disease development.

P.A1.02.14

Investigating the Role of Tetraspanin 5 in Tumour Associated Macrophages

M. Moamin[1]*, P. Monk*[1]*, J. Saint Pol*[2]*, E. Rubinstein*[2]*, M. Muthana*[1]*;*
[1]UNIVERSITY OF SHEFFIELD, Sheffield, United Kingdom, [2]Inserm/Universite Paris-Sud, Paris, France.

Rationale: Tumour-associated macrophages (TAMs) actively promote all aspects of tumour initiation, growth, and metastasis. Following exposure to tumour-derived factors we see a significant increase in TSPAN5. We **hypothesise** that TSPAN5 plays a role in supporting the pro-tumour activities of TAMs
Methodology: qPCR was used to determine the expression of TSPAN5 in human monocytes, monocyte derived macrophages (MDMs) and MDMs cultured in tumour-conditioned medium. Expression of TSPAN5 was also confirmed at the protein level. Accell siRNA knockdown was performed to determine the role of TSPAN5 in MDM phenotype and function. NextSeq500 was performed to identify how TSPAN5 influences gene expression in BMDMs prepared from C57Bl/6 wild-type (WT) and TSPAN5 knockout (KO) mice.**Findings:** TSPAN5 was differentially expressed by human monocytes and MDMs. Following differentiation from monocytes to MDMs, the expression of TSPAN5 was significantly reduced, however culturing MDMs in tumour-conditioned medium significantly increased the expression of TSPAN5. This expression was also evident in TAMs located in patient derived breast cancer tissue (n=20). Interestingly, this accounted for 44% of TAMs. Knockdowns of TSPAN5 enhanced MDM migration and cell clustering and analysis of NextSeq500 (n=3) revealed significant changes in genes involved in cell adhesion (Fn1, Cdh11, Lamb1, Cyr61, Postn and Wisp2) and immune regulation (PTGS2, GPX8 and Crip2) in BMDMs from $TSPAN5^{KO}$ mice compared to WT mice. **Conclusion:** TSPAN5 is expressed by TAMs and this may regulate important macrophage properties. **Future studies** will focus on the role of TSPAN5 in preclinical mammary model in C57BL/6 WT and TSPAN 5^{KO} mice.

P.A1.02.15

Cellular Mechanisms Controlling Surfacing of AICL Glycoproteins, Cognate Ligands of the Activating NK Receptor NKp80

S. Neuss[1]*, Y. Bartel*[1]*, C. Born*[1]*, S. Weil*[2]*, J. Koch*[2]*, C. Behrends*[3]*, M. Hoffmeister*[3]*, A. Steinle*[1]*;*
[1]Institute for Molecular Medicine, Goethe University Frankfurt, Frankfurt am Main, Germany, [2]Institute for Tumor Biology and Experimental Therapy, Georg-Speyer-Haus, Frankfurt am Main, Germany, [3]Institute of Biochemistry II, Goethe University Frankfurt, Frankfurt am Main, Germany.

AICL glycoproteins are cognate activation-induced ligands of the C-type lectin-like receptor NKp80 which is expressed on virtually all mature human NK cells. NKp80-AICL interaction stimulates NK cell effector functions such as cytotoxicity and cytokine secretion. Notably, AICL and NKp80 are encoded by adjacent genes in the natural killer gene complex (NKC) and are co-expressed by human NK cells. Whereas AICL is intracellularly retained in resting NK cells, exposure of NK cells to pro-inflammatory cytokines induces AICL surfacing and susceptibility to NKp80-mediated NK fratricide. Here, we characterize molecular determinants of AICL glycoproteins causing intracellular retention thereby controlling AICL surface expression. Cysteine 87 residing within the C-type lectin-like domain (CTLD) not only ensures stable homodimerization of AICL glycoproteins by disulfide bonding, but is also required for efficient cell surface expression of AICL homodimers. In contrast, cytoplasmic lysines act as negative regulators targeting AICL for proteasomal degradation. An atypical and three conventional N-linked glycosylation sites in the AICL CTLD critically impact on maturation and surfacing of AICL which is strictly depending on glycosylation of at least one conventional glycosylation site. However, while extent of conventional N-linked glycosylation positively correlates with AICL surface expression, the atypical glycosylation site impairs AICL surfacing. Stringent control of AICL surface expression by glycosylation is reflected by the pronounced interaction of AICL with calnexin and the impaired AICL expression in calnexin-deficient cells. Collectively, our data demonstrate that AICL expression and surfacing is tightly controlled by several independent cellular post-translational mechanisms.

P.A1.02.16

Impact of obesity on the function and replenishment of hepatic macrophages in acute liver injury-mediated liver regeneration

G. Panetas[1], *L. Assmus*[1], *F. Gondorf*[2], *C. Kurts*[1], *I. Förster*[2], *W. Kastenmüller*[1], *Z. Abdullah*[1];
[1]*Institute of Experimental Immunology, Bonn, Germany,* [2]*Molecular Immunology and Cell Biology, Life and Medical Sciences Institute, Bonn, Germany.*

Obesity often leads to chronic inflammatory liver diseases such as non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Increasing number of obese patients show a significant risk factor for drug-induced liver injuries and poor liver regeneration after resection of liver mass. Macrophages are key players, supporting tissue regeneration after injury. In this study, the composition of liver myeloid cells, hepatic macrophage function and replenishment were investigated after liver injury, in a model of obesity-induced inflammation. The composition of myeloid cells in livers of obese mice, was investigated with flow cytometry. Sorted liver macrophages from mice on high fat diet, were cultured with apoptotic cells or bacteria to determine their phagocytic capacity *in vitro.* Furthermore, acute liver injury was induced with carbon tetrachloride (CCl_4) and serum levels of liver enzyme ALT were measured. Liver macrophages 30 days post injury, were stained with liver resident and bone marrow derived macrophage markers for flow cytometry and immunofluorescence. The composition of myeloid cells in the livers of mice on high fat diet, have reduced Kupffer cell number and increased infiltration of neutrophils, inflammatory macrophages and monocytes. Additionally, hepatic macrophage show impaired phagocytosis of both dead cells as well as bacteria *in vitro*. Furthermore, in the liver of mice on high fat diet, hepatic macrophages were unable to acquire a Kupffer cell phenotype following acute liver injury. Identifying the cellular and molecular mechanisms by which obesity might affect the function and phenotype of macrophages in liver regeneration will potentially open avenues to novel therapeutic concepts.

P.A1.02.17

Differential cellular programs of conditionally immortalized yolk sac and bone marrow macrophages

C. Schulz[1], *C. Stremmel*[1], *S. Elhag*[1], *A. Zehrer*[1], *B. Walzog*[1], *H. Häcker*[2], *E. Hammer*[3];
[1]*Ludwig-Maximilians-University, Munich, Germany,* [2]*Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, United States,* [3]*Department of Functional Genomics, University of Greifswald, Greifswald, Germany.*

Introduction: Yolk sac (YS) hematopoiesis gives rise to tissue macrophages in many organs. However, the cellular program of these macrophages is incompletely understood. Methods/Results: We conditionally immortalized hematopoietic precursors of YS and bone marrow (BM) origin using estrogen-inducible Hoxb8, and differentiated them into mature macrophages. We than compared in detail their cellular identity and functions. Using proteome and gene expression analysis, we found that macrophages derived from BM progenitors developed a phenotype reminiscent of classically activated macrophages, whereas macrophages differentiated from YS progenitors displayed an increased abundance of anti-inflammatory proteins. Further, we identified differences in macrophage metabolism and functions. Conclusion: Our findings suggest that, when compared under standardized conditions ex vivo, distinct molecular programs are present in YS vs BM macrophages. This adds a novel perspective to the regulation of macrophage functions and could provide opportunities for their therapeutic Manipulation.

P.A1.02.18

Towards a better understanding of lung interstitial macrophages identity

J. Schyns[1,2], *D. Pirottin*[1,2], *F. Bureau*[1,2,3], *T. Marichal*[1,2,3];
[1]*Laboratory of Cellular and Molecular Immunology, GIGA-Research, University of Liège, Liège, Belgium,* [2]*Faculty of Veterinary Medicine, University of Liège, Liège, Belgium,* [3]*Walloon Excellence in Life Sciences and Biotechnology (WELBIO), Wallonia, Belgium.*

Lung interstitial macrophages (IM), constituting the non-alveolar lung macrophage compartment, have been shown to exhibit tolerogenic properties at steady-state by inhibiting the ability of dendritic cells to induce allergic type 2 responses against inhaled aeroallergens. Recently, several reports have provided experimental evidence that IM represent a heterogeneous population in the steady-state lung, and encompass at least two subpopulations, each of them likely carrying their own identity, e.g., phenotype, localization, differentiation program, and function. In order to assess the heterogeneity of IM in an unbiased way, droplet-based single-cell RNA sequencing experiments were performed on the IM pool, revealing three subpopulations of IM at the steady-state. Differentially expressed genes among the subpopulations allowed us to find specific surface markers that identify these phenotypically and functionally distinct subpopulations by flow cytometry, a crucial step to assess the identity of IM, and how such identity is imprinted by the local environment to fulfill the functional needs of the lung mucosa.

P.A1.02.19

IFN-γ-induced STAT3 activation as an alternative pathway in acute myeloid leukemia

G. Tunali, *D. Yoyen-Ermis, G. Esendagli;*
Hacettepe University Cancer Institute, Department of Basic Oncology, Ankara, Turkey.

Introduction: Even though IFN-γ is implicated in anti-tumor immunity, immunoregulatory pathways can simultaneously induce upon exposure to this cytokine. In addition to signal transducer and activator of transcription 1 (STAT1), which is main pathway that leads to cytostatic and immune-provoking effects of IFN-γ, STAT3 can also be activated. STAT3 promotes cancer cell growth, survival and immunosuppression. Here, we investigated the influence of IFN-γ exposure on STAT3 induction in acute myeloid leukemia (AML) cells.
Methods: AML cell lines (THP-1, U937, HL-60) were treated with all-trans retinoic acid (ATRA) or 1α,25-dihidroxyvitamin D3 (D3) to modulate their differentiation status. Maturation of AML cells were assessed by cytology and flow-cytometric immunophenotyping. Bone marrow samples from AML or myelodysplastic syndrome patients and $CD11b^+$ myeloid cells from healthy donors were also studied. Cells were stimulated with recombinant IFN-γ for different time periods. Phospho-STAT3 (pSTAT3), total-STAT3 (tSTAT3), pSTAT1 and tSTAT1 levels were assayed by Western-Blot.
Results: STAT3 was efficiently induced with transient IFN-γ stimulation and pSTAT3 levels were compatible to that of pSTAT1. Hence, all samples studied were responsive to IFN-γ. This effect was augmented when AML cells previously underwent ATRA or D3 maturation conditions. STAT3 pathway was transiently activated in healthy myeloid cells but long-term (48h) exposure to IFN-γ had a positive feedback effect on pSTAT3 and tSTAT3 levels in AML cells.
Conclusion: In contrast to its anti-tumor actions, IFN-γ can effectively stimulate STAT3 in AML cells, supporting immunosuppressive characters and pro-survival activities. Targeting STAT3 in AML may complement immunotherapies which are related to IFN-γ-mediated immunity.

P.A1.02.20

Monocytes bearing G551D-CFTR mutation present a high RANK/MCSF co-expression: first evidence of a facilitated osteoclastogenesis in cystic fibrosis patients?

M. Jourdain[1], *D. Abdallah*[1], *C. Guillaume*[1], *N. Ronan*[2], *Y. McCarthy*[2], *E. Flanagan*[2], *B. J. Plant*[3], *J. Jacquot*[1], ***F. Velard***[1];
[1]*EA 4691 BIOS, Reims, France,* [2]*University College Cork National University of Ireland, Cork Cystic Fibrosis Center, Cork, Ireland,* [3]*University College Cork, Dept. of Respiratory Medicine, Cork, Ireland.*

Bone fragility and low bone mineral density often affect children and young adults with cystic fibrosis (CF) disease. Due to the presence of CFTR in monocytes, we hypothesized that it may impact monocyte differentiation and activation in osteoclasts. Osteoclast precursors fuse and differentiate to form bone-resorbing multinuclear osteoclasts upon stimulation by two essential factors, the M-CSF and the RANKL.
We examined the expression level of M-CSFR and RANK receptors on blood monocytes from G551-D CF patients by flow cytometry, prior to and at nine and twelve months after receiving CFTR potentiator ivacaftor.
Compared to healthy controls, our first set of data demonstrates higher level of a double $M\text{-}CSFR^{high}/RANK^{high}$ subpopulation on monocyte of G551-D CF patients, which was reduced by *in vivo* ivacaftor treatment. Moreover, we examined *ex vivo* differentiation and activation of healthy monocytes into osteoclasts for a 21-days period with/or without the addition of Inh-172 drug, an inhibitor of CFTR chloride channel activity. Interestingly, multinuclear osteoclasts derived from Inh172-treated healthy monocytes were largest, more adherent, and were prone to generate large pits and trenches of dentin resorption. In addition, multinuclear osteoclasts derived Inh172-treated healthy monocytes released reduced level of bioactive lipid mediator sphingosine 1-phosphate (S1P), a key mediator in the directed migration of osteoblast/osteoclast precursors attached to the bone surface. Altogether, these data highlight the critical regulatory role of CFTR in M-CSFR and RANK receptors expression in monocytes, and suggest CF bone disease as a new, cell-type-monocyte dysfunction disease. Vaincre la Mucoviscidose and Vertex Inc. provided funding supports.

P.A1.02.21
Long-term effects of microglial depletion on tau pathology and spatial memory

*H. Lund[1,2], **K. Zhu**[1,2], M. Pieber[1,2], M. Ohshima[3], K. Blomgren[3], X. Zhang[1,2], R. A. Harris[1,2];*
[1]Department of Clinical Neuroscience, Applied Immunology and Immunotherapy, Karolinska Institutet, Stockholm, Sweden, [2]Center for Molecular Medicine, Karolinska Hospital at Solna, Stockholm, Sweden, [3]Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden.

A hallmark of Alzheimer's disease pathology is neurofibrillary tangles comprising hyper-phosphorylated tau. Microglia are resident myeloid cells of the CNS that are implicated in neuro-inflammatory and neurodegenerative disorders. To investigate the reciprocal relationship between microglia and tau pathology we first characterized the microglial response in a mouse model of progressive tau accumulation (hTau mice). We did not detect changes in microglia surface receptor expression, proliferation, cytokine production, morphology or transcriptional profile in aged hTau mice indicating a lack of pathogenic microglia responses to tau aggregation. To assess the direct impact of microglia on tau pathology and associated neurological deficits we developed a protocol for long term microglial depletion in CX3CR1^{CreER}R26^{DTA} mice and crossed them with hTau mice. We then depleted microglia for 3 months which resulted in exacerbation of spatial memory function. These results indicate that microglia have a neuroprotective role during Alzheimer's related tau pathology.

P.A1.02.22
Newborn babies have high number of immature *monocytes expressed CD116* in peripheral blood than adults

***M. Y. Koker**, S. Özsoy, B. Bingöl, S. Kütük, Ç. Karaca;*
Erciyes medical school, Kayseri, Turkey.

Monocytes are able to differentiate to dendritic cells (DCs) under inflammatory situations. Different monocyte subsets show distinct inflammatory cytokine profiles and differentiation potential under steady-state and inflammatory situations. The major subset of monocytes consists of CD14-high CD16-negative (CD14++CD16−). Commited dendritic cell originated from immature monocytes. In humans (hpre-CDC) that develops from committed DC progenitors (hCDPs) in the BM. We have measured the number of immature monocytes (pre-CDC) with CD34+CD38+ CD116+ expression by flow cytometry with acquisition of a million cells from peripheral blood in 10 newborn and 10 adults. To determine the physiological distribution of hpre-CDCs in humans, we examined, peripheral blood, of newborn and adult for small numbers of pre-CDCs travel through the blood and replace cDCs in the peripheral organs, maintaining homeostasis of the highly dynamic cDC pool. Monocyte-derived circulating short-lived pre-CDCs are high in newborn (mean:57 cells/million cells) than adults (mean:7 cells/million cells). We assume that any organ includes epithelial cells, endothelial cells, fibroblasts, stromal cells, and hematopoietic cells are a source of GM-CSF secretion. Circulations of CD116+ short-lived pre-CDCs undergo maturation when going through the vascular environment with high GM-CSF secretion to microenviroment.

P.A1.02.23
Ssu72 phosphatase regulates tissue-resident macrophage function

***E. Park**[1], S. Lee[1], C. Lee[1,2];*
[1]Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon 16419, Korea, Republic of, [2]Department of Health Sciences and Technology, SAIHST, Sungkyunkwan University, Seoul 06351, Korea, Republic of.

Dynamic alterations of phosphorylation state of many cellular signaling-mediated proteins regulate their molecular and cellular fates. Ssu72 is dual protein phosphatase that can act upon tyrosine or serine/threonine residues and transcription/RNA-processing factor. Ssu72 has been characterized as an RNA polymerase II carboxy-terminal domain phosphatase that specifically catalyses serine-5-p dephosphorylation. Recently, we reported that Ssu72 functions as a cohesin-binding phosphatase and interplays with Aurora B kinase for regulation of duplicated sister chromatid separation, and that the deletion of hepatocyte specific Ssu72 led to the development of a high incidence of fatty liver diseases. Ssu72 is known to be expressed in a tissue-specific manner, and we found that Ssu72 expressed in adipose tissue, especially strongly in brown adipose tissue. In this aspect, we generated conditional knock out mice which Ssu72 is deleted specifically in adipose tissue and found that the deficiency of Ssu72 leads to BAT dysfunction compared to wild type mice. Interestingly, we observed not only dramatically reduction of macrophage population but also defective M2 macrophage generation in Ssu72-deficient BAT. Thus, we further generated myeloid cell specific Ssu72 knockout mouse model. This study will include the physiological relevance of Ssu72 loss-of-function in tissue-resident macrophage.

P.A2.01 Immune development and aging from the cradle to the grave - Part 1

P.A2.01.01
Differential Recovery Of Intrathymic Microenvironments That Follows Thymus Damage Results In Qualitative Changes In T-cell Reconstitution

***A. Alawam**, A. J. White, W. E. Jenkinson, G. Anderson;*
University of Birmingham, Birmingham, United Kingdom.

Following ablative therapies used for cancer treatment, damage to the thymus disrupts it ability to support T-cell development. This results in delayed T-cell reconstitution and a period of immunodeficiency that leaves patients susceptible to potentially fatal infections. Thus, examining mechanisms that control thymus regeneration, and identifying new approaches to boost thymus recovery, are important in devising new therapeutic strategies to improve immune reconstitution.
We have used sub-lethal irradiation (SLI) in a mouse model of thymic injury, and performed systematic examination of the recovery of both thymocytes and the thymic microenvironment. Following SLI, we find the generation of CD4$^+$CD8$^+$ thymocytes and their CD4$^+$ and CD8$^+$ progeny occurs in two distinct waves. Analysis of early thymic progenitors indicates that while an initial and transient wave of recovery occurs via a radioresistant intrathymic progenitor, a second sustained wave occurs via thymus entry of bone marrow progenitors. Surprisingly, concurrent analysis of the thymic microenvironment indicates cortial thymic epithelial cell numbers remain constant, suggesting they are radioresistant and available to support thymocyte development. In contrast, medullary thymic epithelial cells and dendritic cells are depleted following damage. Consistent with this, recovery of medulla-dependent Foxp3$^+$ regulatory T-cells occurs after the generation of conventional CD4$^+$ thymocytes.
In summary, our findings suggest that following damage, distinct thymic areas show differential recovery kinetics that impact upon the quality of new T-cell production. Ongoing studies are examining whether known regulators of thymus recovery, including KGF and LTβR stimulation, can restore medullary microenvironments to ensure balanced recovery of T-cell development.

P.A2.01.02
Effect of IL-15 cytokine on the adhesion and migration properties of CD4+CD28null T-lymphocytes in rheumatoid arthritis patients

*M. A. Moro García[1], L. García Jartín[1], E. Bueno García[1], A. García Torre[1], P. Castro Santos[2], R. Díaz Peña[2,3], **R. Alonso Arias**[1,2];*
[1]Hospital Universitario Central de Asturias, Oviedo, Spain, [2]Facultad de Ciencias de la Salud, Universidad Autónoma de Chile (Fondecyt Regular 1151048), Talca, Chile, [3]Inmunología, Centro de Investigaciones Biomédicas (CINBIO), Universidade de Vigo, Vigo, Spain.

CD4+CD28null T-lymphocytes are cells with terminal differentiation that appear after repeated antigenic stimulation and are found at high levels in patients with rheumatoid arthritis (RA). The main objective of this work was to study the adhesion and migration capacity of these cells and the effect of the cytokine IL-15 on these properties. The experiments were performed with peripheral blood samples from patients with RA, where the CD4+ T-lymphocytes were isolated. To study the adhesion ability of CD4+CD28null cells CD11a, CD49d, CD44, CCR5 and CX3CR1 molecules were studied by flow cytometry. The basal level of all these molecules was higher in CD4+CD28null T-lymphocytes. Moreover, IL-15 induced a significant increase in CD11a and CD44 in CD4+CD28null T-cells. Cell migration was studied in CD4+ T lymphocytes isolated and cultured in "transwell". Migrated cells were significantly higher in the wells with IL-15 and the majority of these cells were CD4+CD28null. This effect was IL-15 dose and time dependent. We also studied the activation and activity of Rho A, Rac 1 and Cdc42, proteins involved in cell migration. Both the basal levels of Rho A and the activated RhoA, Rac1 and Cdc42 were clearly higher in CD4+CD28null T-cells. In conclusion, CD4+CD28null T-cells exhibited very different migratory and adhesion properties compared to CD4+CD28+ T-cells in AR. This could be interesting when designing different therapeutic targets to try to prevent the migration and accumulation of these cells in locations where they could exert a pathogenic function and thus, slow down the progression of the disease.

P.A2.01.03

Antigen and adjuvant trafficking within and outside of early life lymph nodes

F. Auderset[1,2,3], *A. Rochat*[1,2,3], *P. Fontannaz*[1,2,3], *C. Tougne*[1,2,3], *P. Lambert*[1,2,3], *C. Siegrist*[1,2,3];
[1]*Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland,* [2]*World Health Organization Collaborating Center for Vaccine Immunology, Faculty of Medicine, Geneva, Switzerland,* [3]*Center for Vaccinology, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland.*

A key determinant of B cell responses is the delivery of antigens to B cells and follicular dendritic cells. Small soluble antigens gain access to B cell follicles through the conduit system, whereas larger antigens are captured by the subcapsular (SCS) macrophages lining the sinus of the lymph node (LN) and transferred to follicular B cells. Neonatal immunization is known to elicit poor and delayed germinal center (GC) B cell responses, suggesting suboptimal B cell targeting and activation. Given their role in antigen capture and delivery, we studied the postnatal development of SCS macrophages and show that they develop very slowly after birth, appearing in LNs only at 3 weeks of age. To investigate the consequences of their absence in neonates, we compared the fate of fluorescent small and large antigens by injecting OVA-FITC or PE, respectively, adjuvanted to the ASO3 oil-in-water adjuvant. Surprisingly, we observed $ASO3^+Ag^+$ cells in contralateral non-draining LNs of 1-wk-old and 3-wk-old mice 24 hours post-immunization, independently of the size of the antigen.
This resulted in local activation and proliferation of $CD4^+$ T cells and development of GCs. This process was oil-in-water adjuvant-dependent and likely resulted from the expression of monocyte chemo-attractants in distant LNs. Our data suggest lymph node impaired barrier and systemic diffusion of vaccine formulations containing oil-in-water adjuvants. Overall, ontogeny exerts a profound influence on antigen/adjuvant retention and diffusion, which should be taken into account when designing vaccine formulations considered for use in early life.

P.A2.01.04

Acetylation of PLZF regulates the lineage specification of iNKT cells

C. joseph[1,2], *j. klibi*[1,2], *M. Delord*[3,4], *V. parietti*[3,5], *M. Pla*[1,6], *B. Lucas*[1,7], *C. Chomienne*[1,6], *A. Toubert*[1,2], *F. Guidez*[1,6], ***k. benlagha***[1,2];
[1]*inserm, paris, France,* [2]*INSERM, UMR-1160, Institut Universitaire d'Hématologie, Paris, France, Université Paris Diderot, Sorbonne Paris Cité, Paris, France,* [3]*Université Paris Diderot, paris, France,* [4]*Plateforme de Bio-informatique et Bio statistique, Institut Universitaire d'Hématologie, Université Paris Diderot, Sorbonne Paris Cité, Paris, France,* [5]*Département d'Expérimentation Animale, Institut Universitaire d'Hématologie, Paris, France ; Université Paris Diderot, Sorbonne Paris Cité, Paris, France,* [6]*INSERM, UMR-1131, Institut Universitaire d'Hématologie, Paris, France, Universite Paris Diderot, Sorbonne Paris Cité, Paris, France,* [7]*Institut Cochin, Centre National de la Recherche Scientifique UMR8104, INSERM U1016, Université Paris Descartes, Paris, France.*

The transcription factor PLZF (promyelocytic leukemia zinc finger) encoded by the BTB domain containing 16 (*Zbtb16*) gene is expressed during the development of invariant natural killer T (iNKT) cells to direct the acquisition of their effector program. In this study we addressed the role of PLZF acetylation in iNKT cells by analyzing the development these cells in mice expressing a constitutive acetylated form of PLZF (PLZFon mice). We found that the constitutive activation of PLZF repressed the development of all iNKT cell subsets (NKT1, NKT2, and NKT17). This developmental block is intrinsic to iNKT cells as assessed by reconstitution experiments. In these mice iNKT cells are blocked at the $CD44^{low}$ to $CD44^{high}$ transition, do not upregulate IL-17Rb, and produce mostly IL-4 and IL-13, indicating that the block occurs at an early precursor stage. Finally, we found in normal mice that the acetylase EP300, known to induce PLZF acetylation in vivo, is expressed at the early $CD44^{low}$ stage 1 of development, indicating that a similar activation could take place during normal iNKT cell development. In addition, we found that the deacetylase HDAC3 and SIRT1, also PLZF partners, are expressed at the $CD44^{high}$ stage, indicating that PLZF suppression could be alleviated at the subsequent developmental stage. Overall, our study reveals a non-expected suppressive rather than activating role of PLZF while promoting iNKT cell development and proposes a model where a tight control of its acetylation/deacetylation during developmental stages regulates lineage specification of this population.

P.A2.01.05

The adjuvant LT-K63 overcomes neonatal limitations in germinal center induction by circumventing the induction of suppressive regulatory cells

S. P. Bjarnarson[1,2], *A. A. Aradottir Pind*[1,2], *G. J. Magnusdottir*[1,2], *G. Del Giudice*[3], *I. Jonsdottir*[1,2];
[1]*Faculty of Medicine, School of Health Sciences, University of Iceland, Reykjavík, Iceland,, Reykjavik, Iceland,* [2]*Department of Immunology, Landspitali, the National University Hospital of Iceland, Reykjavik, Iceland,* [3]*GSK Vaccines, Siena, Italy.*

Introduction: High susceptibility to infectious diseases and low vaccine responses characterizes the neonatal and infant period due to immature immune system. Formulating antigens with adjuvants may enhance vaccine immunogenicity and efficacy. T follicular helper (Tfh) cells play an important role in germinal center (GC) reaction, production of antibody secreting cells (AbSCs) and memory B cells, whereas follicular T regulatory cells (Tfr) can suppress Tfh and GC B cells numbers. We evaluated the effects of the adjuvant LT-K63 on Tfh and regulatory cells in relation to its enhanced neonatal vaccine-induced humoral response. Materials and Methods: The frequency of Tfh, Tfr, regulatory T cells (Tregs), IL-10-secreting B cells (B10), GC B cells, plasmablasts and plasma cells, was assessed in 7-28 days old and adult mice, and 4, 8, 14 and 21 days after neonatal parenteral immunization with pneumococcal conjugate (Pnc1-TT) w/wo LT-K63. Vaccine-specific AbSCs and Abs were also measured. Results: Maturation of Tfh, Tfr cells, Tregs and B cells was age-dependent, in neonates a larger fraction of $CD4^+$ T cells and B cells differentiated into IL-10-secreting Tregs or B10 and B10-phenotype differed. Upon immunization LT-K63 enhanced neonatal induction of Tfh compared to vaccine alone and increased the ratio of $T_{FH}/T_{FR,}$ but decreased Treg and B10. Accordingly, LT-K63 enhanced the induction of GC B cells, plasmablasts, plasma cells, vaccine-specific AbSC and Abs. Conclusion: The adjuvant LT-K63 contributes to enhanced T_{FH} differentiation and induction of GC B cells by circumventing the induction of suppressive regulatory cells, contributing to enhanced and persistent vaccine responses.

P.A2.01.06

Telomere length on individual chromosome arms in patients with immunopathology and healthy donors

M. S. Barkovskaya, ***E. A. Blinova****, J. V. Konyahina, A. E. Sizikov, D. V. Demina, V. A. Kozlov;*
Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation.

Telomere length is an important factor characterizing immune system, its decreasing indicates a premature aging in immune-mediated diseases. The distribution of telomere repeats on different chromosomes has an individual telomere profile in humans. The purpose of this study was to estimate the telomere lengths in the individual chromosome arms in patients with rheumatoid arthritis (RA), (n=6, the median age 51.5 (50-54)) and bronchial asthma (BA), (n=6, the median age 43,5 (37-57)) in comparison with healthy donors. Two groups of donors were selected strictly by age and each consisted of 6 human. Written informed consents were obtained from persons enrolled in the study. RA and BA were diagnosed by clinicians according to ACR/EULAR-2010 and GINA-2017.
Metaphase spreads obtained from PBMCs were used. For measurement of the telomere length Q-FISH with $(C_3TA_2)_3$ PNA-probe was applied. The new MeTeLen software was developed to estimate the telomere repeats quantity (http://www.bionet.nsc.ru/en/development/application-development/development-of-a-computer/metelen.html) in metaphase images.
When comparing the telomere length, it was found, that telomeres on p-arms of chromosome 15 in RA and 9 in BA are significantly shorter than in corresponding group of donors ($p< 0.05$, Mann-Whitney U-test). Also the common telomere profiles obtained from groups of patients and donors were evaluated. Analysis revealed a number of identical and distinctive features in distribution of significantly short (9q, 15q, 17p, 22q) and long (3p, Xp) telomeres (Wilcoxon-signed-runk test). This may be consequence of a proliferative stress in the immunopathology or instead a congenital feature, that accelerates immunosenescence and play role in pathogenesis of studied diseases.

P.A2.01.07

Type I Interferons (IFNs) are toxic during neonatal influenza virus infection

A. J. Carey[1], *O. K. Kumova*[1], *A. J. Fike*[1], *Y. M. Mueller*[2], *P. D. Katsikis*[1,2];
[1]*Drexel University College of Medicine, Philadelphia, United States,* [2]*Erasmus University Medical Center, Rotterdam, Netherlands.*

Although Type I IFNs constitute an efficient innate defense mechanism, type I IFN production and IFN receptor (IFNαβR) signaling can also serve to amplify proinflammatory responses, increasing respiratory tract damage. We questioned the role of Type I IFNs during neonatal influenza infection. Three-day old and 8-week old adult C57Bl/6 mice were infected with influenza virus and followed for mortality. Three-day old mice were exquisitely sensitive to influenza virus infection and exhibited high mortality and viral load. To test the role of Type I IFNs, IFNαβ receptor deficient mice ($IFN\alpha\beta R^{-/-}$) and age-matched C57/Bl6 mice were infected on the third day of life. Surprisingly, the $IFN\alpha\beta R^{-/-}$ mice had an improved survival rate of 80%, compared to the C57/Bl6 survival rate of 15%. We further confirmed this deleterious effect of type I IFN by treating neonatal mice intranasally with IFNβ 24 hours after influenza infection. Treating with IFNβ after influenza infection accelerated and increased mortality in the neonatal mice ($p<0.05$). When neonatal mice, however, were treated with IFNβ before influenza virus infection on days 1 and 2 of life, and then infected on day of life 3, mice were protected ($p<0.01$). Thus, IFNβ treatment timing in relation to neonatal influenza infection is critical for the function of IFN. Our studies reveal that Type I IFNs are toxic in the neonate in the setting of a viral infection.

P.A2.01.08

Immunomodulatory activity of Lactobacillus isolated from human milk and baby stools

***M. C. de Almagro**, G. Cifuentes, M. García, J. A. Moreno, J. Jiménez, M. Rodríguez-Palmero;*
Laboratorios Ordesa, Sant Boi, Spain.

During the first moths of life, babies have an immature immune system. Breastmilk contains different immunomodulatory compounds that help the infant fight infections and favors a proper maturation of the immune system. Among these breastmilk components bacteria can be found. Probiotics are bacteria regarded as "good bacteria" due to their health benefits when used correctly. To identify new functional probiotics, a proprietary library of *Lactobacillus* isolated from human milk and stools from babies exclusively breastfed, was screened for immunomodulatrory activities. First, probiotics safety was assessed by measuring the cytotoxicity produced by the bacterial lysate as well as the bacterial supernatant in HT29 and THP1 cells. *Lactobacillus* ability to modify the levels of 11 cytokines was measured by Luminex and ELISA in basal conditions and upon LPS or TNFα treatment, obtaining several candidates with antiinflamatory and tolerogenic characteristics (IL-10 secretion and TNFα decrease upon challenge). Among those candidates, two strains with good growth properties up to the pilot scale, were selected. Therefore, two *Lactobacillus* strains isolated from baby stools with inmunomodulatory activity which could potentially be used in baby formula have been found. Support: this project is supported by Centro para el Desarrollo Tecnológico Industrial (CDTI)

P.A2.01.09

VDJ-recombination shapes the clone-size distribution of naive T-cells

***P. C. de Greef**[1], T. Oakes[2], B. Gerritsen[3], J. Heather[2], R. Hermsen[1], B. Chain[2], R. J. de Boer[1];*
[1]Utrecht University, Utrecht, Netherlands, [2]University College London, London, United Kingdom, [3]Yale School of Medicine, New Haven, United States.

The clone-size distribution of the human naïve T cell receptor (TCR) repertoire is unknown, and measuring it is not feasible because of the vast repertoire diversity. We report a strong relation between the frequency of TCR alpha- and beta-chains of naive T-cells in blood samples from adult volunteers and the probability of these sequences to be generated by V(D)J-recombination in the thymus. This strong relation is unexpected because in adults, only a small fraction of naive T-cells is produced by the thymus, while the vast majority are a result of peripheral division. To examine if VDJ-recombination probabilities can indeed explain the frequency differences between TCRs in the naive compartment, we developed simple mathematical models describing naive T-cell dynamics, and compared their predictions with sequencing data. We establish the presence of a small fraction, but a large number, of TCR-sequences that are abundant and frequently generated by the thymus (i.e. the alpha- or beta-chains have a high VDJ-recombination probability). These results demonstrate an unexpectedly large role for VDJ-recombination probabilities in shaping the naïve clone-size distribution, casting doubt on the role that cognate signals play in determining clone-sizes of naive T-cell repertoires.

P.A2.01.10

Young serum and its-derived extracellular vesicles potentially rejuvenate aged T cell immunity & immune tolerance (Supported by NIH R01AI121147 to D-M. S.)

***S. Dong-Ming**, J. Oh, W. Wang;*
University of North Texas Health Science Center, Fort Worth, United States.

Aging results in thymic involution, with declined output of naïve T cells resulting in accumulation of exhausted $CD28^{-neg}$ T cells, involved in immunosenescence; and increased output of self-reactive T cells, related to development of self-reactivity-resulted chronic inflammation. Both conditions establish a risk for increased morbidity and mortality in the elderly. Therefore, it is of great importance to develop rejuvenating strategies able to attenuate these. A promising "rejuvenation factor" present in young blood has been found to be able to make aged neuron "younger". However, it is uncertain whether and how young serum-derived factors work on aged immune system. Herein, we tested rejuvenation in aged human (>70YO) peripheral blood mononuclear cells (PBMCs) with young human serum in humanized mouse model, and in the naturally-aged mouse (>16MO) atrophied thymus with young mouse serum-extracted extracellular vesicles(EVs)/exosomes, in which full of epigenetic regulators are encapsulated. We determined young human serum, but not PBS, was able to reduce $CD28^{-neg}$ cells in CD4 and CD8 subsets, with reduction of global methylation, and increased cell numbers and activation of Bim^+ cells. We also found young murine serum-derived EVs, but not non-EV supernatant, were able to attenuate inflammation in both the periphery and central nerve system of old mice, attributed to partially reversed thymic involution and improved function in thymocyte negative selection. Our results in rejuvenation of T cells on both peripheral immunosenescence and central immune tolerance serve as an impetus to encourage us to determine the underlying mechanism, which is hypothesized due to young serum-provided epigenetic intervention.

P.A2.01.11

Distinct pattern of differentiation and responsiveness of chicken $CD8\alpha\alpha^+\gamma\delta$T cells

***M. Gu**, K. Yu, Y. Kye, B. Park, T. Park, S. Han, C. Yun;*
Seoul National University, Seoul, Korea, Republic of.

The aim of the present study was to investigate phenotypically distinct subset of γδT cells in the periphery and, more importantly, their function. In chickens, CD8 expression subdivides peripheral γδT cells into 3 subsets ($CD8^-$, $CD8\alpha\alpha^+$, and $CD8\alpha\beta^+$ γδT cells). Interestingly, unlike other subsets, $CD8\alpha\alpha^+$ γδT cells were absent in the thymus while they are present in all other organs examined. It is important to note that $CD8\alpha\alpha^+$ γδT cells did not convert into other subsets when stimulated with PMA/IL-2 whilst other subsets were able to become $CD8\alpha\alpha^+$ γδT cells. Furthermore, $CD8\alpha\alpha^+$ γδT cells in the periphery exhibited high expression of CD25, CD28 and CD44 compared to other subsets. These results indicated that $CD8\alpha\alpha^+$ γδT cells are most likely the form of terminally differentiated subset. To further investigate the response to innate and TCR stimuli, we prepared supernatant from total splenocytes treated with LPS (LPS supernatant), which contains major inflammatory cytokines (IL-1β, IL-6 and IL-23). γδT cells were treated with the LPS supernatant and/or αCD3/28 monoclonal antibodies (for TCR stimulation). We found that LPS-induced mediators induced proliferation of $CD8\alpha\alpha^+$ γδT cells which was mostly CD5 negative, while TCR stimulation induced proliferation of $CD5^+CD8\alpha\alpha^+$ γδT cells. These results suggested that proliferation of $CD8\alpha\alpha^+$ γδT cells with a distinct CD5 expression pattern displayed distinct response to innate (inflammatory cytokines) and adaptive (TCR stimulus) signal. Collectively, chicken $CD8\alpha\alpha^+$ γδT cells have heterogeneity of responsiveness to innate and TCR stimuli, which may help for investigating and understanding their functional role in infection and inflammation.

P.A2.01.12

Lineage specification of promyelocytic zinc finger-expressing innate iNKT cells is controlled by strength of TCR signaling

*c. joseph[1,2], **j. klibi**[1,2], l. amable[1,2], l. comba[1,2], A. Cascioferro[3,4], M. Delord[5,6], V. Parietti[5,7], c. Lenoir[8,9], S. Latour[8,9], B. Lucas[1,10], C. Viret[1,11], A. Toubert[1,2], k. benlagha[1,2];*
[1]inserm, paris, France, [2]INSERM, UMR-1160, Institut Universitaire d'Hématologie, Paris, France, Université Paris Diderot, Sorbonne Paris Cité, Paris, France, [3]Institut Pasteur, paris, France, [4]Unité de Pathogénomique Mycobactérienne Intégrée, Institut Pasteur, 75724 , France., Paris, France, [5]Université Paris Diderot, paris, France, [6]Plateforme de Bio-informatique et Bio statistique, Institut Universitaire d'Hématologie, Université Paris Diderot, Sorbonne Paris Cité., Paris, France, [7]Département d'Expérimentation Animale, Institut Universitaire d'Hématologie, Paris, France, Université Paris Diderot, Sorbonne Paris Cité., Paris, France, [8]Imagine Institut, paris, France, [9]Laboratory of Lymphocyte Activation and Susceptibility to EBV infection, Inserm UMR 1163, Paris, France, Imagine Institut, Université Paris Diderot, Sorbonne Paris Cité, Paris, France, [10]Institut Cochin, Centre National de la Recherche Scientifique UMR8104, INSERM U1016, Université Paris Descartes, Paris, France, [11]CIRI, International Center for Infectiology Research, Université de Lyon, Lyon, France; INSERM, U1111, Lyon, France; CNRS, UMR5308, Lyon, France.

Invariant Natural Killer T (iNKT) cells are innate-like T lymphocytes, the development of which depends on the transcription factor PLZF. We aimed in this study to assess the contribution of the canonical TCR Va14-Ja18 alpha chain in driving iNKT1, 2, and 17 developmental programs. Analysis of iNKT cell development in conventional Va14-Ja18 transgenic (Tg) mice, where the Va14-Ja18 transgene is expressed as early as the $CD4^-CD8^-$ double negative stage, shows that the Va14-Ja18 chain does not favor the entry of iNKT cells into a specific developmental program. However, we found that iNKT cell specification is affected in CD4-Vα14-Ja18 Tg mice, where the transgene is controlled by a CD4 promoter retarding its expression at the $CD4^+CD8^+$ double positive stage. In these mice, we observed an increase in the $T\text{-}bet^-ROR\gamma t^-$ subset that we found to represent early iNKT cell precursors rather than NKT2 cells. These cells have a block in the transition from the developmental $CD44^{low}$ stage 1 to $CD44^{high}$ stage 2 and a defect in acquiring IL-17Rb at the $CD44^{low}$ stage 1, revealing an earlier developmental block. Analysis performed at the transcription and protein levels showed a reduced expression of Egr-2 and its target gene PLZF in developing iNKT cells from CD4-Va14-Ja18 Tg mice. Moreover, we observed that these cells perceived a weaker TCR signal that is likely at the origin of their altered downstream TCR signaling and subsequent developmental defect. Overall, our study highlights TCR signal strength, and not Va14-Ja18 chain, as an important regulator of iNKT cell lineage specification.

P.A2.01.13

Antibody secreting plasma cells persist for decades in human intestine

O. J. B. Landsverk[1], *O. Snir*[2], *R. B. Casado*[1], *L. Richter*[1], *J. E. Mold*[3], *P. Réu*[3,4], *R. Horneland*[5], *V. Paulsen*[5], *S. Yaqub*[6], *E. M. Aandahl*[5,7], *O. M. Øyen*[5], *H. S. Thorarensen*[8], *M. Salehpour*[9], *G. Possnert*[9], *J. Frisén*[3], *L. M. Sollid*[10], *E. S. Bækkevold*[1], *F. L. Jahnsen*[1];

[1]Department of Pathology and Centre for Immune Regulation, Oslo University Hospital-Rikshospitalet and the University of Oslo, Oslo, Norway, [2]Department of Immunology, Oslo University Hospital-Rikshospitalet and the University of Oslo, Oslo, Norway, [3]Department of Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden, [4]Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal, [5]Department of Transplantation Medicine, Oslo University Hospital-Rikshospitalet, Oslo, Norway, [6]Department of Gastrointestinal Surgery, Oslo University Hospital-Rikshospitalet, Oslo, Norway, [7]Centre for Molecular Medicine Norway, Nordic EMBL Partnership, University of Oslo and Oslo University Hospital-Rikshospitalet, Oslo, Norway, [8]Department of Informatics, University of Oslo, Oslo, Norway, [9]Department of Physics and Astronomy, Ion Physics, Uppsala University, Uppsala, Sweden, [10]Department of Immunology, Centre for Immune Regulation and KG Jebsen Coeliac Disease Research Centre, University Hospital-Rikshospitalet and the University of Oslo, Oslo, Norway.

Plasma cells (PCs) produce antibodies that mediate immunity after infection or vaccination. In contrast to PCs in the bone marrow, PCs in the gut have been considered short-lived. Here, we studied PC dynamics in the human small intestine by cell-turnover analysis in organ transplants and by retrospective cell birth dating measuring carbon-14 in genomic DNA. We identified three distinct PC-subsets: A $CD19^+$PC-subset was dynamically exchanged, whereas of two $CD19^-$PC-subsets; $CD45^+$PCs exhibited little and $CD45^-$PCs no replacement, and had a median age of 11 and 22 years, respectively. Accumulation of $CD45^-$PCs during ageing and the presence of rotavirus-specific clones entirely within the $CD19^-$ PC-subsets support selection and maintenance of protective PCs for life in human intestine

P.A2.01.14

Characterisation and Development of Unconventional T cells

H. Koay[1,2], *C. V. Nguyen-Robertson*[1,2], *M. N. Souter*[1,2], *S. J. Reddiex*[1,2], *C. F. Almeida*[1,2], *C. Harpur*[1,2], *S. Su*[3], *D. Zalcenstein*[3], *C. Seillet*[3], *J. Rossjohn*[4,5], *Y. d'Udekem*[6], *I. E. Konstantinov*[6], *O. T. Nguyen*[1,2], *K. Kedzierska*[1,2], *T. Cheng*[7], *A. A. Eltahla*[8], *I. Van Rhijn*[7], *G. T. Belz*[3], *A. P. Uldrich*[1], *S. Naik*[3], *F. Luciani*[8], *S. J. Williams*[9], *B. D. Moody*[7], *D. I. Godfrey*[1,5], ***D. G. Pellicci***[1,2];

[1]University of Melbourne, Melbourne, Australia, [2]Peter Doherty Institute, Melbourne, Australia, [3]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [4]Department of Biochemistry and Molecular Biology, Monash University, Clayton, Melbourne, Australia, [5]Australian Research Council Centre of Excellence for Advanced Molecular Imaging, Melbourne, Australia, [6]Royal Children's Hospital, Parkville, Melbourne, Australia, [7]Brigham and Women's Hospital Division of Rheumatology, Immunology and Allergy, Harvard Medical School, Boston, Massachusetts, United States, [8]School of Medical Sciences, Faculty of Medicine, University of New South Wales, Sydney, Australia, [9]School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Melbourne, Australia.

Most studies on T cells have focused on conventional T cells that respond to peptides from viruses, bacteria and other pathogens. However, the human immune system contains large populations of unconventional T cells that recognise lipid antigens or Vitamin B derivatives presented by CD1 and MR1 molecules, respectively. Surprisingly, little is known about the role of these T cells in human immunity, largely owing to a lack of specific reagents to identify these cells. We have produced CD1 tetramers that contain endogenous mammalian lipid antigens or antigen-specific CD1 tetramers that contain lipid antigens derived from *Mycobacterium tuberculosis* (Mtb). We show that we can clearly identify both endogenous and Mtb reactive CD1 tetramer positive cells from healthy human blood. Moreover, using MR1 tetramers, we identify previously undescribed populations of Mucosal-Associated Invariant T (MAIT) cells within mouse and human thymus. We use a combination of multicolour flow cytometry, RNA-seq and gene deficient mice to characterise these poorly understood unconventional T cell populations and provide novel insight into the molecular mechanisms that underpin their development and function.

P.A2.01.15

Human iNKT cell subsets during thymic development & in the periphery

J. Perroteau[1], *L. Hesnard*[1], *M. Devilder*[1], *L. Gapin*[2], *E. Scotet*[1], *X. Saulquin*[1], *L. Gautreau-Rolland*[1];

[1]Centre de Recherche en Cancerologie et Immunologie Nantes-Angers, Nantes, France, [2]National Jewish Health - University of Colorado, Denver, United States.

Invariant Natural Killer T (iNKT) cells are unconventional T lymphocytes that express both NK receptors and a semi-invariant αβ TCR (T Cell Receptor). This TCR is restricted by the CD1d molecule presenting glycolipid antigens, and among them, αGalactosylCeramid (αGalCer) is a ligand of all iNKT cells. Recently, three principal subsets of iNKT cells have been described in mice, based on transcription factor expression and cytokine production: NKT1, NKT2 and NKT17 cells (Lee et al., 2013). However, the existence of specialized iNKT subsets remains uninvestigated in humans.

We previously studied the thymic maturation of human antigen-specific conventional T cells (Hesnard et al., 2016) thanks to a highly sensitive tetramer-based immunomagnetic cell separation approach (Legoux et al., 2010). Here, we adapted this strategy to enrich human iNKT cells, with fluorescent αGalCer-CD1d tetramers, from human thymuses and peripheral blood. A ten-parametric flow cytometry analysis, comprising transcription factors and membrane antigens, has allowed retracing the development of iNKT cells in humans.

We determined the presence of iNKT cells in the human thymus at a frequency of 1.10^{-6} among CD3+ cells. Most of them were CD4+CD8- and CD4-CD8-, but CD4+CD8+ and CD4-CD8+ subsets were also revealed. The activation status of these subsets showed a classical thymic differentiation with the loss of coreceptor expression over the thymic development. Finally, no functionally specialized iNKT subsets were depicted in the thymus, unlike in periphery. Altogether, these results suggest that human iNKT cells' development follows the kinetic model and that the establishment of distinct subsets is acquired in periphery.

P.A2.01.16

Tumor suppressor BAP1 is essential for thymic development and proliferative responses of T lymphocytes

S. Rutz, *T. Arenzana, A. Seki, C. Eidenschenk, D. Arnott, Z. Modrusan, A. Dey;*

Genentech, South San Francisco, United States.

BAP1 is a ubiquitously expressed nuclear deubiquitinating enzyme studied mostly for its tumor suppressor function. Loss-of-function mutations of BAP1 have been identified in a variety of solid tumor types, and are strongly linked to metastasis and poor prognosis.Surprisingly, we found that tamoxifen-induced BAP1 deletion in adult mice resulted in severe thymic atrophy and complete loss of the T cell lineage. B cell development was also abrogated suggesting a broader function for BAP1 in maintaining the lymphoid, but not the myeloid lineage. BAP1 deficiency resulted in a block at the DN3 stage prior to the pre-T cell receptor checkpoint. Peripheral T cells in CD4.Cre-driven T cell-conditional BAP1 KO mice exhibited a defect in homeostatic and antigen-driven expansion, showed strongly reduced production of Th1 and Th17 cytokines, and were completely protected from T cell-mediated autoimmunity. Deletion of BAP1 resulted in suppression of E2F target genes and defects in cell cycle progression, which was dependent on the catalytic activity of BAP1. Similar to the role of the BAP1 homolog Calypso in Drosophila, loss of BAP1 led to strongly increased global mono-ubiquitination of histone H2A at lysine 119 (H2AK119ub) throughout the T cell lineage, in particular in immature thymocytes. Histone PTM profiling by MS also identified diminished histone H3S10 phosphorylation, a hallmark of mitosis, further demonstrating a crucial role for BAP1 in regulating G2/M transition in T lymphocytes. Our findings uncover a non-redundant epigenetic function for BAP1 in regulating and maintaining proliferative capacity within the lymphoid lineage.

P.A2.01.17

Impaired balance of CD45RA and CD45RO membrane expression of blood T- lymphocytes in children with hepatitis: common features of autoimmune and chronical viral diseases?

E. Semikina[1], *A. Toptygina*[2], *A. Potapov*[1], *E. Kopyltsova*[1], *A. Surkov*[1], *S. Akulova*[1];

[1]) Federal State Autonomous Institution, Moscow, Russian Federation, [2]G.N.Gabrichevsky Research Institute for Epidemiology and Microbiology, Moscow, Russian Federation.

The impaired balance of CD45RA+ and CD45RO+ lymphocytes (lphs) in pediatric patients (pts) with autoimmune diseases can reflect the severity of permanent immune process, but the main reason is unclear: is it the primary immune dysregulation or the result of long-term inflammation? We compared the frequency of significant deviations of CD45RA/CD45RO expression in 25 children with autoimmune hepatitis (AIH) and 25 with chronical hepatitis C (HC), groups were equivalent with ages (7-15 y.o.), genders, and disease activity. The CD45RA and CD45RO membrane expression on peripheral blood lphs was determined with four-color flow cytometry (BD FacsCalibur, Cell-Quest Software, BD Biosciences MultiTest CD45RA/CD45RO/CD3/CD4 & CD45RA/CD45RO/CD3/CD8 MABs). The CD45RA/CD45RO expression was examined in the gates of T-cells (CD3+), T-helpers (CD3+CD8+) and T-cytotoxic lphs (CD3+CD8+) separately . The individual CD45RA/CD45RO balance was compared with previously determined normal age-related levels. The advanced (forestalling to age) CD45RO expression at CD3+ and CD3+CD4+ was found in 6 of AIH pts (24%) and in 7 of HC pts (26%). The advanced CD45RO expression at CD3+CD8+ lphs was found only in 1 HC patient. The proposed negative correlation between age and CD45RA+ lphs and positive correlation between age and CD45RO+ lphs and was proven for CD3+ and CD3+CD4+ gates, not for CD3+CD8+. We suppose that "pre-mature ageing" of some lph's populations can be an unspecific event in chronical immune diseases independently of main pathological reason, but can be useful for long-term follow-up.

P.A2.01.18

The corepressor NCOR1 regulates the survival of single-positive thymocytes

*L. Müller[1], D. Hainberger[1], **V. Stolz**[1], P. Hamminger[1], H. Hassan[2], T. Pregleji[1], N. Bucheron[1], S. Sakaguchi[1], J. Wiegers[3], A. Villunger[3], J. Auwerx[4], W. Ellmeier[1];*
[1]Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria, [2]Dept. of Biochemistry (Shankar Campus), Abdul Wali Khan University (AWKUM), Mardan, Pakistan, [3]Innsbruck Medical University, Biocenter, Division of Developmental Immunology, Innsbruck, Austria, [4]Ecole Polytechnique Fédérale de Lausanne, Laboratory of Integrative and Systems Physiology, Lausanne, Austria.

Nuclear receptor corepressor 1 (NCOR1) is a transcriptional regulator bridging repressive chromatin modifying enzymes with transcription factors. NCOR1 regulates many biological processes, however its role in T cells is not known. Here we show that *Cd4*-Cre-mediated deletion of NCOR1 (NCOR1 cKO^{Cd4}) resulted in a reduction of peripheral T cell numbers due to a decrease in single-positive (SP) thymocytes. In contrast, double-positive (DP) thymocyte numbers were not affected in the absence of NCOR1. The reduction in SP cells was due to diminished survival of NCOR1-null postselection TCRβ^{hi}CD69^{+} and mature TCRβ^{hi}CD69^{-} thymocytes. NCOR1-null thymocytes expressed elevated levels of the pro-apoptotic factor BIM and showed a higher fraction of cleaved caspase 3-positive cells upon TCR stimulation *ex vivo*. However, staphylococcal enterotoxin B (SEB)-mediated deletion of Vβ8^{+} CD4SP thymocytes was normal, suggesting that negative selection is not altered in the absence of NCOR1. Finally, transgenic expression of the pro-survival protein BCL2 restored the population of CD69^{+} thymocytes in NCOR1 cKO^{Cd4} mice to a similar percentage as observed in WT mice. Together, these data identify NCOR1 as a crucial regulator of the survival of SP thymocytes and revealed that NCOR1 is essential for the proper generation of the peripheral T cell pool.

P.A2.01.19

Association of cystatin-3 gene polymorphisms with schizophrenia

***R. Zakharyan**[1,2], V. Hayrapetyan[1], L. Karapetyan[2], A. Arakelyan[1,2];*
[1]Institute of Molecular Biology NAS RA, Yerevan, Armenia, [2]Russian-Armenian University, Yerevan, Armenia.

Schizophrenia (SCZ) is a severe mental disease characterized by interplay between genetic and environmental factors. Despite a number of studies performed in the field of schizophrenia research, the molecular mechanisms of the disorder are still unclear. Previous studies including our own have demonstrated that alterations in immune response are contributory factors for schizophrenia. Cystatins are large group of proteins with diverse biological activities including immune response. The aim of the current study was to investigate the potential association of SCZ with rs3827143 single nucleotide polymorphism (SNP) of the gene (CST3), encoding cystatin-3 protein.
A total of 231 unrelated individuals of Armenian nationality (121 SCZ patients and 110 healthy controls) were genotyped using polymerase chain reaction with sequence-specific primers (PCR-SSP). Distribution of genotypes were checked to correspondence to Hardy-Weinberg equilibrium. Statistical analysis was performed using Pearson's Chi-square test. According to the data obtained, the frequency of the CST3 rs3827143*G allele was significantly lower in the group of SCZ patients compared to controls (p=0.0005, OR=0.52, 95%CI: 0.36-0.76). The same tendency was found for the carriage of the CST3 rs3827143*G minor allele (p=0.0024, 95%CI: 0.39-0.73). These data suggested that the CST3 rs3827143*G genetic variant can be considered as a protective factor against SCZ development, at least in Armenian population. Further studies are required to uncover the complete set of variants contributing to this disorder.

P.A2.01.20

CHMP5 is a quantitative TCR signal sensor required for T cell development

***S. Adoro**[1], J. Shim[2], L. H. Glimcher[3];*
[1]Case Western Reserve University School of Medicine, Cleveland, United States, [2]University of Massachusetts Medical School, Worcester, United States, [3]Dana Farber Cancer Institute and Harvard Medical School, Boston, United States.

A self-tolerant and diverse pool of T-cells is essential for adaptive immunity but the mechanism of generation of these T-cells in the thymus remains the subject of intense investigations. Through still poorly understood posttranslational mechanisms, precursor CD4^{+}CD8^{+} double-positive thymocytes are selected for maturation into the peripheral T-cell pool guided by the affinity of their T-cell receptor (TCR) interaction with self-peptides presented by thymic epithelial major histocompatibility complex molecules. While low-affinity TCR interactions induce 'positive selection' leading to T-cell generation, high-affinity TCR ligands induce 'negative selection' by apoptosis, consequently eliminating potentially self-reactive T-cell clones. How signals emanating from the same receptor can generate such diametrically opposite outcomes continues to perplex immunologists. We have identified the endosomal-sorting complex required for transport protein CHMP5 as a key sensor of TCR signal thresholds that promote thymocyte selection. Notably, thymocyte-specific loss of CHMP5 abolished T-cell development in a manner partly dependent on the ability of CHMP5 to stabilize Bcl-2 proteins in TCR-signaled thymocytes. Absent CHMP5, positively selected thymocytes underwent apoptosis that was partially rescued by genetic deletion of the apoptosis activator *Bim* or by transgenic overexpression of Bcl-2. We found that not only was CHMP5 posttranslationally stabilized by the deubiquitinase USP8, it also functioned as an adaptor to orchestrate normal client protein substrate ubiquitination in developing thymocytes. Collectively, our studies have uncovered an unexpected checkpoint during positive selection mediated by CHMP5 and identify CHMP5 as an essential component of the posttranslational machinery required for T-cell development.

P.A2.01.21

Human MAIT cell count forms a surrogate for general immune health

*S. Danielli, I. Nassiri, E. Mahe, R. Cooper, R. Watson, H. Al-Mossawi, P. Klenerman, **B. P. Fairfax**;*
University of Oxford, Oxford, United Kingdom.

Human mucosal associated invariant T-Cells (MAIT) form a subset of CD8^{+} cells with recombined T-cell receptor (TCR) alpha chains containing the product of *TRAV1-2* with either *TRAJ-33*, *TRAJ-20* or *TRAJ-12*. MAIT cells have a distinct gene expression profile with high expression of *KLRB1* encoding CD161 and *RORC* encoding the transcription factor RORγt. Whilst MAIT cells are conserved from mice to humans, the determinants of number and their gene expression pattern in humans are poorly understood. Using TCR mapped from purified CD8^{+} T-cell RNA-sequencing data (196 samples from 102 individuals) we define a proxy for MAIT count with which we deconvolute a MAIT specific gene signature that out-performs single-cell sequencing data from the same individuals. We replicate previous findings of a rapid decline in MAIT count with age and we further find that MAIT count correlates with other signatures of immune health including T-cell clonality and anti-viral and pro-inflammatory gene expression signatures in NK cells and monocytes, from the same individuals, respectively. Within MAIT cells we find 155 genes whose expression is associated with age, including *AHRR*, *PTPRB* and *GPR15*. We also observe 20 sex associated genes within MAIT cells. Finally, we demonstrate a number of other TCR within CD8+ T-cells are correlated with MAIT count, suggesting shared factors controlling MAIT cell count over life and other subsets.

P.A2.01.22

STRAUSS: Population-wide STudy on Risk of Anti-Ulcer medications for SubSequent anti-allergic drug prescription

***E. Jensen-Jarolim**[1,2], M. Kundi[3], E. Untersmayr[1], I. Pali-Schöll[1,2], B. Reichardt[4], G. Jordakieva[1,2,5];*
[1]Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria, [2]The Interuniversity Messerli Research Institute, Medical University Vienna, University of Veterinary Medicine Vienna, University of Vienna, Vienna, Austria, [3]Center for Public Health, Medical University of Vienna, Vienna, Austria, [4]Sickness Fund Burgenland, Austria , Burgenland, Austria, [5]Department of Physical Medicine, Rehabilitation and Occupational Medicine, Medical University of Vienna, Vienna, Austria.

Background: Anti-ulcer medications, such as proton pump inhibitors (PPI), are amongst the most frequently prescribed drugs in Europe. In recent years, however, evidence for an association between gastric pH modulation and allergic sensitization has been reported. To evaluate this suspected association, we aimed to assess the frequency of prescribed anti-allergy treatment following acid-inhibitors prescription in Austria. **Methods:** Data from health insurance claim records 2009-2013 were assessed in a population-based analysis, covering 8.2 million (approx. 97%) of the Austrian population. Consecutive prescriptions of gastric acid-inhibitors (PPI, H2-receptor antagonists, sucralfate, prostaglandin E2) followed by anti-allergic drugs (antihistamines, desensitization therapy) prescriptions were analysed Austria-wide. As control condition, consecutive prescriptions of other common medications (lipid-modifying and antihypertensive drugs) followed by anti-allergics were analysed in a regional subgroup (Burgenland) controlling for age and gender. **Results:** The specific hazard for the prescription of anti-allergic drugs was doubled in the overall population (1.96 [95%CI:1.95-1.97]) and triplicated in the regional data set [3.07 (95%-CI:2.89-3.27)], after prescription of gastric acid inhibiting drugs ($p<10^{-17}$). These findings were more prominent in women (p<0.001 compared to males). Age and gender adjusted hazard ratio was 2.05 (95%CI:1.91-2.19), and elevated independent of preceding acid-inhibitor type. The risk increased age-dependent from 1.47 (95%CI:1.45-1.49) in <20 year olds, up to 5.20 (95%-CI:5.15-5.25) in >60 year olds. **Conclusions:** We found a highly significant association between acid-inhibiting and subsequent anti-allergic drug prescriptions. These population-wide findings are in line with previous mechanistic and observational studies, in both animals and humans, further supporting a causal relationship between gastric acid-modulation and allergic sensitization.

P.A2.02 Immune development and aging from the cradle to the grave - Part 2

P.A2.02.01
CMV-infection in chronic heart failure patients contributes to a higher inflammatory status

M. A. Moro García, A. García Torre, E. Bueno García, B. Díaz-Molina, J. Lambert, ***R. Alonso Arias****;*
HOSPITAL UNIVERSITARIO CENTRAL DE ASTURIAS, OVIEDO, Spain.

Chronic heart failure (CHF) is characterized by high levels of proinflammatory mediators and disease progression may be a result of the deleterious effects exerted by endogenous cytokine cascades on the heart and the peripheral circulation. CMV-infection is the best known inducer of the differentiation of T lymphocytes in elderly and also contributes to the immunosenescence found in CHF patients. To analyze the association between CMV-serostatus and inflammation in CHF we study 40 patients (age: 55,5±6,9 years), 13 CMV-seronegative and 27 CMV-seropositive. Cytokine levels were quantified using a multiplex system (Luminex) and peripheral blood mononuclear cells (PBMC) were isolated and stimulated in vitro for 72 hours with anti-CD3 and LPS. Higher levels of IL-1b, IL-6, TNF, IL-17A and IL-12p70 ($p<0,05$) were found in the serum of CMV-infected patients compared to CMV-uninfected patients. No differences in IFN-γ, IL-2, IL-4 or IL-10 levels were found. Moreover, inflammation was related not only to CMV-infection, but also to anti-CMV antibody titers which showed positive correlation with IL-1b, IL-6, TNF and IL-17A ($p<0.05$).
When PBMC were stimulated in vitro, significant differences were also found between both groups of patients. PBMC from CMV-seropositive patients produced higher levels of proinflammatory cytokines in response to anti-CD3 treatment, whereas no differences were found in response to LPS stimulation. This could be due to the greater differentiation of CD4+ and CD8+ T-lymphocytes found in CMV-seropositive patients.
In conclusion, inflammation found in CHF patients may be related to dynamics of CMV-infection presumably as a consequence of their effects on T-lymphocyte differentiation.

P.A2.02.02
T-LGL leukemia cells display features of exhausted and senescent T cells

J. L. J. Assmann*, M. J. Kallemeijn, A. W. Langerak;*
Dept. of Immunology ErasmusMC, Rotterdam, Netherlands.

Background: Large granular lymphocyte leukemia is a rare heterogeneous hematological disorder that has a chronic disease course and mostly affects the elderly. LGL leukemia is estimated to account for 2-5% of all chronic lymphoproliferative disorders. Most commonly TCRαβ+ CD8+ T cells are affected, even though in rarer cases the γδ T cell lineage can be affected as well . Chronic (antigenic) stimulation is hypothesized to be one of the key players in disease onset. Since the disease largely presents with come of age, and since immuno-senescence is a well described phenomenon in the ageing individual, here we tried to identify if T-LGL leukemia cells of both lineages present characteristics of senescence and/or exhaustion at phenotypical, functional and gene expression levels.
Results: Based on our observations through 15-color flow cytometry and qPCR analysis, phenotypically and transcriptionally T-LGL leukemia cells were exhausted rather than senescent. On a functional level, the T-LGL cells adopted the senescence-associated secreting phenotype, which is characterized by overproduction of cytotoxins. Additionally, T-LGL leukemia cells showed diminished proliferation capacity and resistance to apoptosis, which is correlated with immuno-senescence.
Conclusion: Collectively, our data indicate that T-LGL leukemia cells adopt the most detrimental characteristics from both senescence and exhaustion, thereby secreting large amounts of cytotoxins without resulting in full replicative senescence and apoptosis, respectively. We further hypothesize that this could eventually result in the accumulation of terminally differentiated, activated, cytotoxic and apoptosis-resistant T-LGL leukemia cells that concomitantly induce heterogeneous disease characteristics in patients.

P.A2.02.03
T cell phenotypes in cytomegalovirus infected young adults are similar to those seen in elderly adults and are associated with reduced vaccine responses

G. Bowyer*[1], N. Venkatraman[1], T. Lambe[1], N. Brenner[2], C. Mair[1], T. Waterboer[2], S. Gilbert[1], A. Hill[1], K. Ewer[1];*
[1]The Jenner Institute, Oxford, United Kingdom, [2]Infection and Cancer Epidemiology, DKFZ, Heidelberg, Germany.

Introduction: CMV has been associated with reduced vaccine responses in both elderly and younger adults, although the underlying mechanisms are currently unclear, particularly in younger adults.
Methods: We conducted a Phase I clinical trial of viral-vectored Ebola vaccine candidates ChAd3-EBO-Z and MVA-EBO-Z in healthy young adults (18-50years). We assessed the impact of CMV serostatus on vaccine-specific T cell and antibody responses. We also compared T cell phenotypes in these adults with CMV- and CMV+ adults aged 60-80years to determine the differential impacts of CMV serostatus and age.
Results: CMV seropositivity was associated with significantly reduced vaccine responses and striking differences in the global T cell repertoire with a shift towards late-differentiated memory T cells expressing CD57 and killer cell lectin-like receptor G1 (KLRG1). The proportion of vaccine-specific $CD4^+$ and $CD8^+$ T cells expressing these markers was significantly higher in CMV+ individuals and negatively correlated with vaccine responses - 0.71% vs 0.27% of the $CD4^+$ and 19.9% vs 4.10% of the $CD8^+$. $CD57^+KLRG1^+$ $CD4^+$ cells were expanded in both CMV+ young and CMV+ older individuals but not CMV- individuals regardless of age.
Conclusions: This study suggests that CMV, which has previously been associated with immunosenescence and reduced vaccine immunogenicity in elderly populations, can impact negatively on vaccine responses in young adults. Expansion of a subset of $CD4^+$ T cells expressing terminal differentiation markers in association with CMV serostatus and not age suggests that CMV acquisition rather than age might be responsible for reduction of some vaccine responses in elderly cohorts.

P.A2.02.04
Landscape of naive T cell repertoire changes with human age

E. Egorov[1,2], S. Kasatskaya[1,3], T. Nakonechanya[1], M. Pogoerlyy[1], M. Shugay[1], D. Chudakov[1,2,3], ***O. Britanova****[1];*
[1]IBCH, Moscow, Russian Federation, [2]Pirogov Russian National Research Medical University, Moscow, Russian Federation, [3]Center for Data-Intensive Biomedicine and Biotechnology, Skolkovo Institute of Science and Technology, Moscow, Russian Federation.

Human aging is associated with profound changes in T cell immunity, compromising our ability to withstand the new challenges including response to infections and vaccination. These changes may further result in the imbalanced immune response leading to nonspecific inflammation provoking neurodegenerative and cardiovascular disorders, increasing risk of cancer development and autoimmune diseases. Prolonged peripheral proliferation could be associated with the functional deficiency of naive T cells that fail to differentiate towards memory phenotype upon a specific antigenic challenge. How uniform is the naïve T cells proliferation on the periphery remains questionable. To shed the light on the nature of ongoing age-related changes, here we focused on the comparative analysis of intrinsic characteristics of TCR repertoires for the bulk naive CD8+, bulk naive CD4+, naive RTE-enriched CD31+CD4+ and naive non-RTE CD4+ T cells derived from peripheral blood of young versus elder healthy donors. We revealed several notable changes in characteristics of T cell repertoire. Characteristics of TCR beta CDR3 repertoires changed significantly in CD4 and CD8, both RTE-enriched and mature naive CD4 T cell subsets. Biochemical characteristics in the middle of TCR beta CDR3 also changed prominently. Relative publicity of CD4 but not CD8 naïve T cells repertoire increased. We propose several explanations for these phenomena, and call for further studies of the mechanisms causing the observed changes and of consequences of these changes in respect of the possible wholes formed in the landscape of naïve T cells TCR repertoire. This work was supported by the Russian Science Foundation project №16-15-00149.

P.A2.02.05
Progressive long-term avidity decline of CMV- but not EBV-specific CD8 T cell clonotype repertoires

B. Couturaud*[1], M. Allard[1], L. Carretero-Iglesia[1], D. E. Speiser[1,2], M. Hebeisen[1], N. Rufer[1,2];*
[1]Lausanne University Hospital Center and University of Lausanne, Epalinges, Switzerland, [2]Ludwig Cancer Research, University of Lausanne, Epalinges, Switzerland.

Efficient T cell responses rely on the TCR-pMHC-CD8 binding avidity that controls all essential T cell functions. However, it still remains unknown whether the TCR-ligand avidity is a determining factor for the clonal selection and evolution of antigen-specific T cells over time. Here, we studied the TCRαβ repertoire composition and selection over a period of 15 years combined with TCR-pMHC-CD8 binding avidity analyses of large panels of CMV- and EBV-specific CD8 T cell clones. We found that the TCRαβ clonotype composition of both CMV- and EBV-specific T cell responses remains remarkably stable during the studied period. Nevertheless, within the CMV-specific clonotype repertoires, we observed the preferential selection and expansion over time of clonotypes of lower TCR-pMHC avidity and higher CD8 binding dependency, correlating with reduced functional capacities. In contrast, the clonal evolution of the EBV-specific clonotype repertoires was highly preserved, with the presence of the same clonotype distribution (i.e. dominant versus sub-dominant, low versus high TCR avidity, CD8 binding-independent versus -dependent) over time. Our results indicate that the TCR-pMHC-CD8 binding avidity represents a major determinant of clonal selection and evolution in long-lasting CMV-specific T cells, consistent with the current concept of clonal senescence of high avidity T cells with aging. However, this is not the case for EBV-specific CD8 T cell repertoires, in which the clonal composition and distribution once established is kept highly constant for at least 15 years. These findings suggest distinct mechanisms regulating the long-term outcome of CMV- versus EBV-specific CD8 T cell responses in humans.

P.A2.02.06

Cigarette Smoke exposure during pregnancy increase the susceptibility of the inflammation process in the central nervous system (CNS) of the offspring

A. C. S. Durão[1], *W. N. Brandão*[2], *N. Ghabdan*[2], *J. S. Peron*[2], *T. Marcourakis*[1];
[1]*Faculty of Pharmaceutical Sciences, São Paulo, Brazil,* [2]*Institute of Biomedical Sciences, São Paulo, Brazil.*

During the implantation phase, the embryo is more vulnerable to external influences such as cigarette smoke, which can increase the risk of fetal developmental delay and immune system abnormalities. This study evaluated the effect of cigarette smoke exposure during pregnancy on an inflammatory response in the CNS of the offspring. C57BL/6 mice were exposed to 3R4F cigarette smoke, or synthetic air, from vaginal plug to offspring birth. At the 3rd day of life, offspring were separated for the following studies: 1) *in vitro*: brains were dissected and a mixed glial culture was prepared. After 21 days, the cells were stimulated with 100 ng/mL LPS or culture medium. After 24h, the pro-and anti-inflammatory cytokines were evaluated by CBA, as well as GFAP, CD11b, CD80 and CD86 by flow cytometry. 2) *in vivo*: animals were challenged with LPS (1 mg/kg) or saline i.p.. After 4h, the mice were euthanized and the CNS removed for PCR analysis. The *in vitro* experiments showed a proliferation of astrocytes and an increase in proinflammatory cytokines in the cells from animals exposed to cigarette smoke and challenged with LPS when compared to the control. *In vivo* the PCR Array showed that mice exposed to cigarette smoke and LPS had decreased expression of the CCR2, MHCII and SOCS1 genes. Our results suggest that exposure to cigarette smoke during pregnancy increases the inflammation response in the CNS of the offspring after an inflammatory stimulus. Financial Support: FAPESP, CNPq

P.A2.02.07

LKB1 expressed in dendritic cells governs the development of thymus-derived regulatory T cells

L. R. Pelgrom[1], *F. Otto*[1], *A. Ozir-Fazalalikhan*[1], *A. Sergushichev*[2], *M. N. Artyomov*[3], *H. H. Smits*[1], ***B. Everts***[1];
[1]*LUMC, Leiden, Netherlands,* [2]*ITMO University, St. Petersburg, Russian Federation,* [3]*Washington University, St. Louis, United States.*

Liver Kinase B1 (LKB1) plays a key role in cellular metabolism by controlling AMPK activation. However, its function in dendritic cell (DC) biology has not been addressed. Here, we found that in vitro cultured murine DCs that lack LKB1 displayed impaired AMPK, but heightened mTOR activation, and expressed higher levels of costimulatory molecules and CCR7 upon TLR stimulation, show stronger migratory capacity, resulting in stronger T cell priming capacity *in vitro* and, following adoptive transfer, *in vivo.* Surprisingly however mice with a DC-specific deficiency in LKB1 (CD11c-cre / LKB1-fl/fl [CD11cΔLKB1]) displayed reduced effector T cell responses in models of immunization. Instead, CD11cΔLKB1-mice harbored a dramatically expanded population of helios$^+$Foxp3$^+$ thymus-derived functional regulatory T cells (tTregs), already during steady state, which provides a mechanistic basis for the impaired response to immunization. Consistent with this Treg-dominated immune signature, CD11cΔLKB1-mice failed to develop proper anti-tumor immunity and were resistant to induction of allergic asthma. Mechanistically, we found that specifically thymic CD11b$^+$ cDCs from CD11cΔLKB1-mice displayed an enhanced ability to promote tTreg differentiation which correlated with elevated antigen processing and presentation in these cells. Together, our findings identify LKB1 as a key regulator of DC activation thereby governing the development of tTregs and outcome of immune responses.

P.A2.02.08

Association of human obesity with PD-1 exhaustion phenotype in T cells

R. Flores-Mejia[1], *J. I. Leon-Pedroza*[2,3], *A. Monroy-Guzman*[4], *O. Rodriguez-Cortes*[5], *R. Chacon-Salinas*[2], *E. Hernandez-Leon*[6], *E. Calderon-Austria*[6], *C. V. Gaona-Aguas*[6], *S. A. Estrada-Parra*[2];
[1]*SEPI, Escuela Superior de Medicina. Instituto Politecnico Nacional, México, D.F., Mexico,* [2]*Depto. Inmunologia, Escuela Nacional de Ciencias Biologicas. Instituto Politecnico Nacional, México, D.F., Mexico,* [3]*Hospital General de Mexico, Ciudad de Mexico, Mexico,* [4]*Hospital General de Mexico, México, D.F., Mexico,* [5]*SEPI, Escuela Superior de Medicina. Instituto Politecnico Nacional, México, D.F., Mexico,* [6]*Escuela Superior de Medicina. Instituto Politecnico Nacional, México, D.F., Mexico.*

Lymphocytes are able to display in chronic inflammatory conditions, a diminished functional state known as exhaustion. Protein PD (Programmed Death)-1. inhibits activation and proliferation of T cells. Besides, obesity creates a chronic inflammatory state associated with insulin resistance (IR), but if this inflammation is capable of generate lymphocyte exhaustion is unknown. Patients and methods. Adults between 18 and 60 years old, with normal weight, overweight and obesity, and without other immunologic conditions or acute or chronic infection previously diagnosed, were recruited at the Diabetes Prevention Clinic at General Hospital of Mexico. All of them give their informed consent to participate and were studied through clinical examination, 75g oral glucose tolerance test and metabolic panel. Expression of PD-1 in peripheral blood lymphocytes, both CD45RO and CD45RA was conducted through flow cytometry. Statistical analysis was done with Student's t, one way ANOVA, MANOVA, chi-squared and Pearson's correlation. Results: Obesity and IR were associated with higher serum of C reactive protein, total leukocyte count, lymphocyte count and CD4 T cells. Individuals with obesity had higher counts of lymphocytes CD4+CD45RA+PD1+ than those without obesity (1.3 SD 0.5 vs. 11.4 SD 9.7; p = 0.02). Those subjects less insulin sensitive (Matsuda index < 4.0) had more T cells CD4+CD45RO+PD1+ than those having Matsuda index > 4.0 (28.9 SD 11.9 vs. 12 SD 3.1, p = 0.034). Conclusions: IR is associated with an inflammatory phenotype with activated lymphocytes expressing more PD-1, while obesity is associated with a phenotype in which naïve lymphocytes express more PD-1. SIP20180760

P.A2.02.09

Consequences of prenatal glucocorticoid treatment on the development of allergic and autoimmune diseases

A. Gieras[1], *C. Gehbauer*[1], *D. Perna-Barrull*[2], *L. Glau*[1], *I. Diepenbruck*[1], *S. A. Joosse*[3], *N. Kersten*[1], *S. J. Bremer*[1], *D. E. Zazara*[4], *P. C. Arck*[4], *F. R. Stahl*[5], *M. Vives-Pi*[6], *E. Tolosa*[1];
[1]*Department of Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany,* [2]*Immunology Division, Germans Trias i Pujol Research Institute and Hospital, Universitat Autonoma de Barcelona, Spain,* [3]*Department of Tumor Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany,* [4]*Department of Obstetrics and Prenatal Medicine, Laboratory for Experimental Feto-Maternal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany,* [5]*Institute of Clinical Chemistry and Laboratory Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany,* [6]*Immunology Division, Germans Trias i Pujol Research Institute and Hospital, Universitat Autonoma de Barcelona, Badalona, Spain.*

Introduction:
Prenatal betamethasone is routinely administered to pregnant women at risk of preterm delivery to improve survival of the newborn. Even though glucocorticoids induce a plethora of effects on immune cells there is little known whether this treatment might affect the development of the offspring's immune system. Here we investigate the immunological long-term consequences of prenatal glucocorticoid administration in different mouse models of disease.
Methods: Time-pregnant females were treated with betamethasone one day before birth. Experimental allergic asthma was induced in C57BL/6 offspring by sensitization and challenge with ovalbumin. The kinetics of postnatal seeding of peripheral immune organs was assessed in the same strain. The TCR repertoire and development of autoimmunity was monitored in the adult offspring of NOD (diabetes) and MRL/MpJ-Fas^{lpr}/J (lupus) mice.
Results: Delayed seeding of T and B cells was observed in offspring of betamethasone-treated animals. In the NOD model, prenatal betamethasone treatment decreased the frequency of pathogenic T cells and the incidence of type 1 diabetes. In contrast, in the lupus-prone MRL/lpr strain, prenatal glucocorticoids induced changes in the T cell repertoire that resulted in more autoreactive cells. No differences were observed in allergic airway response after ovalbumin sensitization and challenge in prenatally-treated animals.
Conclusion: Prenatal steroid treatment, by inducing changes in the T cell receptor repertoire, has unforeseeable consequences on development of autoimmune disease. Our data should encourage further research to fully understand the consequences of this widely used treatment.
This work was supported by the DFG (KFO296) and the Studienstiftung des deutschen Volkes (Promotionsstipendium).

P.A2.02.10

Aging causes loss of CD122-mediated responsiveness to interleukin-2 in CD8$^+$T cells

D. Pieren, N. Smits, ***T. Guichelaar****;*
National Institute for Public Health and the Environment, Bilthoven, Netherlands.

Interleukin-2 is fundamental to supporting both responsiveness of effector CD8$^+$ T cells (Tc) and suppression by CD25$^+$Foxp3$^+$ regulatory T cells (Treg). Aging has a debilitating impact on the immune system, as marked by the loss of responsiveness of Tc and impaired interleukin-2 production. Moreover, expression of CD122 among Tc and frequencies of Tregs rise. Studies in young mice showed interleukin-2 to favour Tc responses when directed to the CD122 of its receptor, or to favour Treg functionality when directed preferentially to CD25 of the interleukin-2 receptor. Towards unraveling interleukin-2 functionalities at old age, we questioned whether interleukin-2 would reverse the defective Tc response at old age when favouring interleukin-2 binding to CD122 or preventing interleukin-2 binding to CD25. We supplemented anti-CD3-stimulated spleen cell suspensions of young and aged mice with interleukin-2 in the presence of antibodies blocking interaction specifically with CD25 or CD122. Activation of T cells was measured by proliferation and expression of CD69 using flow cytometry. Our study on directing exogenous interleukin-2 to CD122 or CD25 shows that interleukin-2 promotes activation of young Tc cells via CD122, but interleukin-2 never promotes responsiveness of Tc at old age. Moreover, blocking of CD25 enhanced interleukin-2-mediated Tc activation at young age, suggesting a suppressive role for CD25$^+$ Tregs. This modulatory effect was absent at old age. Thus, hyporesponsiveness to interleukin-2-mediated triggering of CD122 contributes to impaired activation of CD8$^+$ T cells at old age. Insight in dysfunctional responsiveness to interleukin-2 may improve our understanding of weakened immunity occurring among the elderly.

P.A2.02.11

Immunomodulation by the transcription factor FOXO3 and its pharmacological activation

J. Hartwig[1], *F. Sotzny*[1], *S. Bauer*[1], *J. Kurreck*[2], *C. Skurk*[3], *C. Scheibenbogen*[1];
[1]*Institute for Medical Immunology, Berlin, Germany,* [2]*University of Technology, Berlin, Germany,* [3]*Department of Cardiology, Berlin, Germany.*

Introduction: FOXO3 is a transcription factor crucial in regulation of cell metabolism, stress resistance and immunity. It is regulated by posttranslational modifications. However, mechanistic insight in FOXO3 effects on immune cell function is still limited. Therefore, the effect of FOXO3 on immune cell function as well as a pharmacological modulation of FOXO3 (e.g. metformin) will be studies in this project. Finally, different SNPs within the FOXO3 gene famous for their association with aging will be analyzed regarding their functional influence on immune cells. Material & Methods: AMPK activation (pT172) was analyzed by Western Blot. AMPK mediated phosphorylation of FOXO3 (pS413) was determined using Flow Cytometry (FC). Reactive oxygen species (ROS) production (DCFH-DA) and cytokine response (TNFα, IFN-, IL-10) was analyzed by FC. mRNA expression of FOXO target genes was assessed by qRT-PCR. Allelic discrimination PCR was used for FOXO3 SNP genotyping. Results: Preliminary data show an AMPK and FOXO3 activation under metformin treatment. This is confirmed by a repression of the FOXO3 target PCK2. Furthermore, Immune cells treated with metformin show reduced ROS and pro-inflammatory cytokine production. In addition, the anti-inflammatory cytokine IL-10 is increased under metformin. An association of three different gain-of-function SNPs in the FOXO3 gene, which were recently published to be associated with aging did not show a functional influence on cytokine or ROS production. Conclusion: Taken together, the results indicate that FOXO3 can be activated with metformin leading to an anti-inflammatory phenotype which might explain its anti-aging effect. DFG sponsored project

P.A2.02.12

Differential effect of cytomegalovirus infection with age on the expression of CD57, CD300a, and CD161 on T-cell subpopulations

F. Hassouneh[1,2], *N. Lopez-Sejas*[1], *C. Campos*[1], *B. Sabchez-Correa*[2], *R. Tarazona*[2], *R. Solana*[1], *A. Pera*[1];
[1]*Instituto Maimónides de Investigación Biomédica de Córdoba -Universidad de Córdoba, Cordoba, Spain,* [2]*Immunology Unit, University of Extremadura, Cáceres, Spain.*

Introduction: Immunosenescence is a progressive deterioration of the immune system with aging. It affects innate and adaptive immunity limiting the response to pathogens and to vaccines. Chronic cytomegalovirus (CMV) infection is probably one of the major driving forces of immunosenescence and its persistent infection results in functional and phenotypic changes to the T-cell repertoire. **Methods:** we analyzed the effect of CMV-seropositivity and aging on the expression of CD300a and CD161 inhibitory receptors and CD57 marker on CD4+ and CD8+ T-cell subsets. **Results:** regardless of the T-cell subset, CD57−CD161−CD300a+ T-cells expand with age in CMV-seropositive individuals, whereas CD57−CD161+CD300a+ T-cells decrease. Similarly, CD57+CD161−CD300a+ T-cells expand with age in CMV-seropositive individuals in both subsets and CD57−CD161+CD300a− T-cells decrease in CD8+ but not in CD4+ T-cells. Besides, in young individuals, CMV latent infection associates with the expansion of CD57+CD161−CD300a+ and CD57−CD161−CD300a+ CD4+ and CD8+ T-cells. Moreover, in young individuals, CD161 expression on T-cells is not affected by CMV infection. Changes of CD161 expression were only associated with age in the context of CMV latent infection. Besides, CD300a+CD57+CD161+ and CD300a−CD57+CD161+ phenotypes were not found in any of the T-cell subsets studied, indicating that in the majority of T-cells, CD161 and CD57 do not co-express. **Conclusions:** our results show that CMV latent infection impact on the immune system depends on the age of the individual, highlighting the importance of including CMV serology in any study regarding immunosenescence. Work supported by grant PI13/02691 from I+D+I National program 2010-2013 and co-funded by "ISCIII-Subdireccion General de Evaluacion" and FEDER

P.A2.02.13

Investigation of intraperitoneal trafficking of lymphocytes in immunodeficient mice

R. Kugyelka, *L. Prenek, A. Lehmann, K. Olasz, T. Berki, P. Balogh, F. Boldizsár;*
University of Pécs, Department of Immunology and Biotechnology, Pécs, Hungary.

The zeta chain-associated protein of 70 kDa (ZAP-70) plays a key role in T cell development and signalling. ZAP-70 homozygous knockout (ZAP-70$^{-/-}$) mice have no mature T cells in their peripheral lymphoid organs and blood. Previously we have shown that the adoptive transfer of wild-type thymocytes reconstitutes this immunodeficiency. We have found that the intraperitoneal (i.p.) route of administration is the most efficient, so we investigated the mechanism of lymphocyte trafficking after i.p. injections.
We used a single i.p. injection to deliver CFSE-labeled donor thymocytes to ZAP-70$^{-/-}$ recipients. We sacrificed animals after various time points and investigated the cellular composition of omentum, peripheral and mesenteric lymph nodes, and spleen. In the mesentery and omentum we analysed the localization of CFSE^{+} donor cells; we also investigated the role of various adhesion molecules (selectins, integrins) and chemokines in the process.
We have found that after i.p. injection donor thymocytes leave the peritoneum and form aggregates in the mesentery along lymphoid vessels and in the omentum, however no donor-originated cells were visible in peripheral or mesenteric lymph nodes. The aggregates found in the omentum are most likeley located in milky spots. Our results suggest that the omentum is an important location for thymocyte entry from the peritoneum in immunocompromised mice, as well.
To gain further insight into the peritoneal trafficking we plan to investigate the possible alterations in trafficking after i.p. injection under different contitions (inflammation, stromal deficiency).
Funding: OTKA K101493; EFOP-3.6.1.-16-2016-00004; GINOP 2.3.2-15-2016-00050; KA-2015-23; BO/00086/12/5; 716/180/2014/KIF; KA-POSTDOK-12-05

P.A2.02.14

Characterization of human dendritic cells from elderly patients stimulated *in vitro* with viral proteins from hamster polyomavirus

A. Müller[1], *N. Maier*[1], *S. Lüth*[2], *W. Dammermann*[2], *K. Hanack*[1];
[1]*University of Potsdam, Institute of Biochemistry and Biology, Potsdam, Germany,* [2]*Brandenburg Medical School Theodor-Fontane, University Hospital Brandenburg, Center of Internal Medicine II, Brandenburg an der Havel, Germany.*

Introduction: Dendritic cells act as highly potent antigen-presenting cells and stimulate T- and B lymphocyte activation to drive immune responses. In elderly persons this potency is strongly reduced due to immunosenescence which leads to insufficient immune responses after vaccination. Increasing age is affecting innate as well as adaptive immune processes, e.g. phagocytic activity, antigen presenting capacity or antigen-specific activation of T- and B lymphocytes. To investigate this further *in vitro*, dendritic cells of young as well as elderly persons were stimulated with viral antigens and characterized in terms of their antigen-specific activation.
Methods: Human monocytes of young and elderly persons were isolated and differentiated *in vitro* to naive dendritic cells using GM-CSF and IL-4. Antigen activation was performed by adding a viral antigen (VP1) in different concentrations (2.5-20 µg/mL). *In vitro* activated dendritic cells of both groups were analyzed and compared by flow cytometry, immunofluorescence and interleukin production.
Results: Human dendritic cells could be activated antigen-specifically *in vitro* by using an antigen concentration of 10 µg/mL as optimum. The expression of different activation markers could be demonstrated and correlated with the production of proinflammatory cytokines. *In vitro* generated dendritic cells of elderly persons showed a reduced activation and capacity to stimulate naive T cells.
Conclusions: There is a high clinical need for improved vaccines to treat elderly persons. The characterization of antigen-specific responses *in vitro* could lead to a better development of vaccine formats in the future.

P.A2.02.15

Oxidative stress and inflammation support the accumulation of highly differentiated T cells in the bone marrow in old age and negatively correlate with Diphtheria antibody titers in the periphery

L. Pangrazzi[1], *E. Naismith*[1], *A. Meryk*[1], *K. Trieb*[2], *B. Grubeck-Loebenstein*[1];
[1]*University of Innsbruck, Innsbruck, Austria,* [2]*Welsklinikum, Wels, Austria.*

Aging induces a basal level of inflammation throughout the body, a condition known as "inflammaging", which contributes to immunosenescence. It has been demonstrated that memory T cells and long-lived plasma cells home to bone marrow niches, well organized structures which promote the survival of these cells. CD4^{+} and CD8^{+} effector memory T cell survival is promoted by IL-7 and IL-15 while maintenance of long-lived plasma cells is supported by APRIL and IL-6. IL-7 is important for long-lived memory T cells while IL-15 is mostly important for highly differentiated T cells, accumulation of which is associated with mortality in old age. The expression of effector memory cell and proinflammatory factors were investigated in bone marrow mononuclear cells (BMMCs) using qPCR and FACS, finding that, with age, IL-7 and APRIL decrease while IL-15, IL-6, TNF, IFNγ and IL1β increase. Incubation of BMMCs with ROS scavengers N-acetylcysteine and vitamin C reduced the levels of both cytokines in this cell population. Furthermore, proinflammatory molecules promoted the accumulation of highly differentiated CD28^{-} T cells, which further support inflammation. A negative correlation was found between ROS, inflammation and senescent CD8^{+} T cells in the BM, and Diphtheria antibody titers in the serum. Our results indicate that oxidative stress and inflammation may contribute to the age-related impairments in the maintenance of immunological memory. Highly differentiated and senescent CD8^{+} T cells in the BM may impair the maintenance of long-lived plasma cells, leading to reduced production of antibody in the periphery as a consequence.

P.A2.02.16

Defective DNA repair contributes to the aging-related accumulation of a CD25^{Low} regulatory T cell population

***D. K. J. Pieren**, N. A. Smits, M. E. Dollé, T. Guichelaar;*
National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands.

Aging has a detrimental impact on T cells. Regulatory T cells (Tregs) accumulate with age and have therefore gained attention in studies on aging. However, the driving force behind the accumulation of Tregs has remained undefined. Gradual accumulation of DNA damage is one of the biological factors that are fundamental to the process of aging. We here aimed to define aging-related accumulation of Tregs and questioned whether defective DNA repair would drive the accumulation of Tregs.
We defined aging of Tregs in wild-type mice at young and old age, and in genetically modified young mice that express a dysfunctional form of the DNA repair protein *excision repair cross-complementing group 1* (Ercc1$^{-/\Delta 7}$). *Ercc1$^{-/\Delta 7}$* is known to cause accelerated development of numerous aging-related pathologies and a reduced lifespan. We phenotypically analyzed CD4$^+$Foxp3$^+$ Tregs with a broad panel of aging markers by flow cytometry and defined subpopulations among these cells using cluster analysis by dimensionality reduction (tSNE).
Multidimensional analysis revealed elevated numbers of Tregs in young *Ercc$^{-/\Delta 7}$* mice and in old wild-type mice compared to young wild-type mice. The major subpopulation comprising this elevated number of Tregs in both naturally aged wild-type mice and in *Ercc1$^{-/\Delta 7}$* mice could be discerned uniquely by low CD25 expression. Furthermore, these CD25^{Low} Tregs show high expression of aging-related markers PD-1 and CD44.
Thus, our study shows that deficiency of DNA repair accelerates the aging-related accumulation of Foxp3$^+$ Tregs. This accumulation is mainly due to the rise of a CD25^{Low} subpopulation.

P.A2.02.17

Deacetylases transcript and protein expression in primary T cell in the context of ageing

***G. M. Toma**[1], D. Quandt[2], B. Seliger[1];*
[1]Martin-Luther University, Halle-Wittenberg, Institute of Medical Immunology, Halle (Saale), Germany, [2]Martin-Luther University, Halle-Wittenberg, Institute of Anatomy, Halle (Saale), Germany.

The capacity of the immune system to protect the organism declines with age, this is observed in the increased susceptibility to infections and decreased efficiency of vaccination. Postranslational modifications of proteins (PTMs) contribute to this by the accumulation of misfolded proteins. One of the most abundant PTMs is acetylation. This process is regulated by: acetyltransferases (ex: p300) and deacetylases. Deacetylases are historically called histone deacetylases (HDACs), but their range of substrates is much wider. The 3rd class of deacetylases is called Sirtuins (SIRT). SIRT1, 3 and 6 have been shown to have a role in the ageing process. Our aim is to analyze if the deacetylases expression levels change during ageing. For this purpose, CD4+ and CD8+ T cells have been sorted magnetically from PBMCs of healthy blood donors from 2 age groups (< 30 yo and > 60 yo) and have been stimulated with plate bound aCD3 and soluble aCD28 for 48h, then the transcript expression of HDACs and Sirtuins has been assessed by qPCR and the protein expression by flow cytometry. Additionally, the constitutive transcript expression of CD4+ and CD8+ T cells has been compared to non-T cells. Sirtuin expression was found higher in T cells, while Sirtuin and p300 expression was decreased in aged CD4 T cells. These results will be linked to proliferation assays and flow cytometry analysis of the T cell subsets. The results of our study will provide insights into the role of deacetylases in the ageing T cells.

P.A2.02.18

Comparative analysis of B-cell repertoires induced by live yellow fever vaccine in young and middle aged dinors

***M. A. Turchaninova**[1], A. N. Davydov[2], A. S. Obraztsova[3,4], M. Lebedin[1], D. Staroverov[1], E. M. Merzlyak[1], O. V. Britanova[1], D. M. Chudakov[1,2,4];*
[1]Institute of Bioorganic Chemistry, Moscow, Russian Federation, [2]CEITEC, Masaryk University, Brno, Czech Republic, [3]Lomonosov State University, Moscow, Russian Federation, [4]Skolkovo Institute of Science and Technology, Moscow, Russian Federation.

Aging is associated with a dysregulation of immune function and age-related changes are reported in many cell populations. These changes include attenuated response to vaccines, an exhausted immune repertoire displaying a substantially lower diversity of T cell receptors as well as impaired antigen-driven selection mechanisms. Recent advances in next-generation sequencing technology have allowed us to perform high-resolution characterization of the antibody repertoire, and of the changes that occur following vaccination in different ages donors. In present study we employed 5'RACE UMI-based full length error-free immunoglobulin profiling to compare plasma cell antibody repertoires in young and old donors vaccinated with live yellow fever vaccine. Our analysis has revealed age-related differences in the responding antibody repertoire ranging from distinct IGH CDR3 repertoire properties to differences in somatic hypermutation profiles and antibody lineage tree structure. Young vaccinated individuals respond with a repertoire containing significantly longer CDR3, implying potentially higher sequence diversity. Elder individuals tend to respond to a new challenge with IGH variants carrying shorter CDR3s with higher content of hydrophobic and strongly interacting amino acid residues. Clonal lineage structure analysis reveals that elder individuals have higher total number of newly acquired hypermutations. But at the same time in elder individuals, replacement-to-silent ratio among the newly acquired unique somatic hypermutations was significantly lower compared to the young donors. Overall, our findings suggest that younger individuals respond with a more diverse antibody repertoire and employ a more efficient somatic hypermutation process than elder individuals in response to a newly encountered pathogen. The work was supported by the grant RSF 14-14-00533.

P.A2.02.19

Resting calpain activity as the key factor in human T cell genesis, function and aging

***J. M. Witkowski**[1], A. Mikosik[1], M. Stosio[1], J. Ruszkowski[1], J. E. Frąckowiak[1], K. Ruckemann-Dziurdzińska[2], A. Daca[2], E. Bryl[2], J. Foerster[3], I. Haponiuk[4], A. Le Page[5], T. Fulop[5];*
[1]Department of Pathophysiology, Medical University of Gdańsk, Gdańsk, Poland, [2]Department of Pathology and Experimental Rheumatology, Medical University of Gdańsk, Gdańsk, Poland, [3]Department of Clinical and Social Gerontology, Medical University of Gdańsk, Gdańsk, Poland, [4]Department of Pediatric Cardiac Surgery, Pomeranian Traumatology Center, Gdańsk, Poland, [5]Faculty of Medicine and Health Sciences, Research Center on Aging, Graduate Program in Immunology, University of Sherbrooke, Sherbrooke, QC, Canada.

Introduction: Calpains and their inhibitor calpastatin form a self-regulatory calpain-calpastatin system (CCS) of limited proteolysis controlling proliferation and apoptosis. Their role for proliferation and aging of human T cells and for thymocyte differentiation was assessed here together with some mechanistic aspects of their maintenance and function.
Methods: Peripheral blood mononuclear cells were obtained from healthy young, elderly and centenarians, while thymocytes were obtained from infants undergoing heart surgery. Cells were left untreated or treated with specific membrane-permeable calpain inhibitors. Lymphocyte proliferation was assessed by flow cytometry using VPD450 dividing cell tracking. Amounts and activities of calpains, as well as the amounts of calpastatin and of phosphorylated NFκB, PLCγ, and p56Lck were assessed in different T cell and thymocyte populations by flow cytometry. Expression of the CCS genes and of some relevant miRNAs identified by the NGS was performed by qRT-PCR.
Results: Calpains are differently active in all populations of T lymphocytes and thymocytes. In the periphery, this activity is necessary for proliferation and cytokine secretion and associated with adequate phosphorylation of signaling molecules. It is sustained by constitutive expression of the CANP-1, CANP-2 and CAST genes correlated with certain miRNAs. With aging, the amounts and activities of calpains change in parallel with impaired proliferation and cytokine secretion, but are maintained at "young" levels in the centenarian T cells.
Conclusion: Constitutive activity of calpains in resting thymocytes and T cells seems indispensable for appropriate differentiation and function of these cells and is modified during aging.

P.A2.02.20

The strategy of the human memory B cell response changes throughout life

***R. Carsetti**[1], E. Piano Mortari[1], O. Grimsholm[2], A. Aranburu[2];*
[1]Bambino Gesù Children Hospital IRCCS, Rome, Italy, [2]Department of Rheumatology and Inflammation Research, Gothenburg, Sweden.

Immunological memory, including memory B cells (MBCs), plasma cells (PC) and their antibodies, is generated by the reaction to infection and vaccination, and protects us from re-infection. Two populations of memory B cells have been described, switched and IgM memory B cells, that execute different and non-interchangeable functions. We have shown before that, whereas switched memory B cells are mostly generated in the germinal centers at all ages, IgM memory B cells can be distinct in three types with different developmental history: innate, remodelled and germinal-center-derived IgM memory B cells. CD27 is the cell surface marker able to identify most MBCs in human. The intensity of CD27 expression changes with age. We now show that, independently of the expressed immunoglobulin isotype, in infants MBCs express low levels of CD27 (CD27^{dull}). In the adult most MBCs are CD27^{bright}. CD27dull and CD27bright MBCs represent two distinct and sequential MBC developmental stages. Stringent Ag-driven pressure selects CD27dull into the CD27bright MBC pool. Our results identify the actors and the strategy of the human MBC response throughout life and give the rationale for the design of age-tailored vaccination protocols.

P.A2.02.21

***In vivo* dynamics of thymic T cell differentiation revealed by a new Timer approach**

A. D. Paduraru[1], *D. Bending*[1], *T. Crompton*[2], *M. Ono*[1];
[1]*Imperial College London, London, United Kingdom,* [2]*University College London, London, United Kingdom.*

It is a central interest in thymus biology to understand how temporally dynamic molecular mechanisms control thymic T cell development *in vivo*. It is therefore important to clarify the temporal sequences of thymic T cell differentiation, but this is difficult due to a lack of tools to analyse the temporal dynamics of differentiating T cells.
Since thymic T cell differentiation is driven by T cell receptor (TCR) signalling, we recently established a new tool, Timer of cell kinetics and activity (Tocky) and developed a Timer reporter for a TCR downstream gene, *Nr4a3*. Timer protein spontaneously changes emission spectrum from blue to red, enabling investigations of the temporal dynamics of molecular and cellular events following TCR signalling *in vivo*.
We first determined the decay rates of Timer-blue and -red fluorescence in order to establish *Nr4a3*-Tocky as a quantitative tool to investigate TCR signal dynamics. Timer-blue fluorescence had a half-life of 4 hours, while Timer-red one was over 5 days. Therefore, blue fluorescence reports real-time transcription, while red fluorescence reports transcriptional history. We then used *Nr4a3*-Tocky to study thymic T cell development. Within $CD4^+CD8^+$ double-positive cells, Timer blue expression occurred mostly in the $CD69^+$ fraction. In $CD4^+$ and $CD8^+$ single-positive cells, Timer was expressed by $GITR^{high}$ cells. Finally, we show the temporal sequences of differentiation events for regulatory T cell differentiation using multidimensional analysis.
In conclusion, we establish *Nr4a3*-Tocky as a new tool for analysing the temporal dynamics of cellular differentiation, and using this, we demonstrate how T cells develop across time during selection processes *in vivo*.
Funding: BBSRC

P.A2.02.22

The role of NF-kappaB transcription factors in virtual memory (Tvm) cells

D. P. Ellis[1], *T. Fulford*[2], *R. Grumont*[1], *R. Slattery*[1], *S. Gerondakis*[1];
[1]*Monash University, Melbourne, Australia,* [2]*Peter Doherty Institute, Melbourne, Australia.*

Virtual memory (T_{VM}) cells are a subset of memory phenotype CD8 T cells that arise naturally in naïve, lympho-replete mice. In contrast to conventional memory CD8 T cells that are generated following an antigen-dependent T cell effector immune response, T_{VM} cells develop from naïve $CD8^+$ T cells in response to cytokine-dependent homeostatic proliferative signals, in particular interleukin (IL)-15.
Utilizing two murine models, we show that the NF-κB transcription factor RelA plays a T cell-intrinsic role in the homeostatic maintenance of T_{VM} cells. Lethally irradiated mice reconstituted with $RelA^{-/-}$ foetal liver-derived hematopoietic stem cells (HSCs) fail to maintain a $RelA^{-/-}$ T_{VM} cell population. This phenotype, which appears in part due to an inability to compete with residual ($RelA^{+/+}$) T_{VM} cells for survival/proliferation signals coincides with reduced expression by $RelA^{-/-}$ T_{VM} cells of the IL-2/15 receptor β-chain (CD122). Conditional inactivation of RelA in T cells ($Lck^{cre}Rela^{fl/fl}$ mice) reveals an issue in maintenance with these mice exhibiting a decline in the T_{VM} cell population from 6 weeks of age to ~50% of normal numbers by 12 weeks, after which this reduced level of T_{VM} cells is maintained throughout adult life. In addition to a reduced expression of CD122, IL-7 receptor α-chain (CD127) expression is lower on $Lck^{cre}Rela^{fl/fl}$ T_{VM} cells when compared to age-matched littermate controls ($Lck^{cre}RelA^{wt/wt}$ mice), a finding suggestive of an IL-7 survival defect. Collectively, these results indicate RelA controls the homeostatic maintenance of T_{VM} CD8 T cells by regulating the expression of cytokine receptors important for T_{VM} cell survival and division.

P.A2.03 Immune development and aging from the cradle to the grave - Part 3

P.A2.03.01

Foliate lymphoid aggregates (FLAgs) - a novel member of serosal lymphoid organoids in mice

P. Balogh[1], *J. Xinkai*[1], *O. Jacobsen*[1], *G. Bedics*[1], *B. Botz*[2,3];
[1]*Department of Immunology and Biotechnology, Pécs, Hungary,* [2]*Department of Pharmacology and Pharmacotherapy, Pécs, Hungary,* [3]*3Molecular Pharmacology Research Group, Szentágothai Research Center, Pécs, Hungary.*

The involvement of non-mucosal lymphoid compartments of the abdominal cavity in the systemic immune responsiveness is almost completely unexplored. In addition to the milky spots within the omentum (MS), small congregates of leukocytes have been described in adipose tissue (fat-associated lymphoid clusters – FALCs). Here we report the identification of a novel form of serosal lymphoid organoids that can efficiently collect intraperitoneal B cells and B-lymphoma cells.
Using a spontaneous high-grade B-cell lymphoma a novel set of lymphoid tissues has been observed, which is characterized by a foliate appearance linked to visceral fat and omental adipose tissue as well as peritoneal membrane, denoted as Foliate Lymphoid Aggregate (FLAg). Their tissue architecture and developmental requirements were studied using whole-mount immunohistochemistry revealing an early macrophage-rich condensation followed by gradual enrichment of B cells, leading to FLAg-like transformation. In Rag-/- mice these structures are absent, whereas the transfer of purified B cells restores them. B cells show a partial segregation from T cells that appear to accumulate in the central regions. These FLAg structures are initially demarked and encapsulated by a rim of macrophages displaying LYVE-1 antigen, and are sensitive for clodronate liposoma-mediated depletion, which also blocks subsequent B-cell entry. In vivo bioimaging tracing reveals that both normal B cells and high-grade B-cell lymphoma cell efficiently home upon intraperitoneal transfer. These data reveal the existence of novel visceral lymphoid tissue formations which may influence abdominal immune surveillance.
Supported by OTKA grant #108429

P.A2.03.02

Early dynamics of mucosal NK cells in the small intestine of infants

A. Sagebiel[1], *F. Steinert*[1], *S. Lunemann*[1], *C. Koerner*[1], *R. Schreurs*[2], *M. Altfeld*[1], *D. Perez*[3], *K. Reinshagen*[3], ***M. J. Bunders***[1];
[1]*Heinrich Pette Institute, Hamburg, Germany,* [2]*AMC, Amsterdam, Germany,* [3]*UKE, Hamburg, Germany.*

Introduction Most of our understanding of the infant NK cells is based on studies investigating blood while there is lack of understanding of the development of NK cell compartment in tissues such as the intestine. **Methods** Immune cells were isolated from human infant and adult small intestines (n=28) and assessed using 18-parameter flow cytometry. **Results** By performing t-SNE analysis including 15 parameters NK cells could be identified as $lin\text{-}CD127^-CD56^+$ cells. In infants, NK cells comprised 33% of the overall lymphocyte population compared to only 5% in adult intestines. The reduction of intestinal NK cells coincided with an increase of T cells, resulting in a log10 decrease of the NK-T cell ratio in adults (p=0.0001). The majority of these large numbers of infant NK cells could be considered as being tissue-resident based on their expression of CD103, and CD49a, while CD69 was higher on NK cells derived from adult tissues. In infants, mucosal NK cells featured a characteristic NK cell phenotype $Eomes^+$, $NKG2A^+$ and higher KIR expression as well as over four times more perforin (53.8%, p=0.011) than adult mucosal NK cells. Stimulation resulted in degranulation ($CD107a^+$) of almost all infant mucosal NK cells compared to adults (p=0.005). In sum, NK cells in infant intestines had a characteristic NK cell phenotype whereas in adults $Nkp44^+CD103^+Eomes^{low}$ persevered. **Conclusion** Large numbers of tissue-resident NK cells are present early in life in the intestinal mucosa. Upon reduction of classical NK cells in the infant mucosa $CD103^+Nkp44^+$ ILC-1-like innate cells in adult epithelium persist.

P.A2.03.03

Human thymopoiesis is controlled by a common genetic variant within the *TCRA-TCRD* locus

E. Clave[1,2], *I. Leston Araujo*[1,2], *C. Alanio*[3,4,5], *E. Patin*[6,7,8], *J. Bergstedt*[9], *A. Urrutia*[3,4,5], *S. Lopez-Lastra*[10,5], *Y. Li*[10,5], *B. Charbit*[3], *M. Hasan*[3], *C. R. MacPherson*[3], *B. L. Melo-Lima*[1,2], *C. Douay*[1,2], *N. Saut*[11,12], *M. Germain*[13,14], *D. Tregouet*[13,14], *P. Morange*[12,11], *M. Fontes*[15,16], *D. Duffy*[3,4,5], *J. P. Di Santo*[10,5], *L. Quintana-Murci*[6,7,8], *M. L. Albert*[3,4,16], *A. Toubert*[1,2,17], *The Milieu Intérieur Consortium;*
[1]*INSERM UMRS 1160, Paris, France,* [2]*Université Paris Diderot, Sorbonne Paris Cité, Paris, France,* [3]*Center for Translational Research, Institut Pasteur, Paris, France,* [4]*Dendritic Cell Immunobiology, Institut Pasteur, Paris, France,* [5]*INSERM UMRS 1223, Paris, France,* [6]*Human Evolutionary Genetics, Institut Pasteur, Paris, France,* [7]*CNRS UMRS-2000, Paris, France,* [8]*Center of Bioinformatics, Biostatistics and Integrative Biology, Institut Pasteur, Paris, France,* [9]*Department of Automatic Control, Lund University, Lund, Sweden,* [10]*Innate Immunity Unit, Institut Pasteur, Paris, France,* [11]*Laboratory of Haematology, La Timone Hospital, Marseille, France,* [12]*Aix Marseille University, INSERM UMRS-1260, INRA U1260, Center for Cardiovascular and Nutrition Research (C2VN), Marseille, France,* [13]*Sorbonne Université, Université Paris 6 / UPMC, INSERM, UMRS-1166, Genomics & Pathophysiology of Cardiovascular Diseases, Paris, France,* [14]*Institute for Cardiometabolism and Nutrition (ICAN), Paris, France,* [15]*International group for data analysis, Institut Pasteur, Paris, France,* [16]*Department of Cancer Immunology, Genentech, South San Francisco, United States,* [17]*Laboratoire d'Immunologie et d'Histocompatibilité, Hôpital Saint-Louis, AP-HP, Paris, France.*

The thymus is the primary lymphoid organ where naïve T cells are generated, however, with the exception of age, the parameters that govern its function in healthy humans remain unknown. Herein, we characterized the variability of thymic function among 1,000 age- and sex-stratified healthy adults of the *Milieu Intérieur* cohort, using quantification of T-cell Receptor (TCR) Excision Circles (TRECs) in peripheral blood T cells as a surrogate marker of thymopoiesis. Age and sex were the only non-heritable factors identified that impact thymic function. TREC levels decreased with age (5% per year, $P=3x10^{-98}$) and were higher in women compared to men (66% increase, $P=2x10^{-15}$).

In addition, genome-wide association study revealed a common variant within the T-cell receptor *TCRA-TCRD* locus, between the *DD2* and *DD3* gene segments (rs2204985, P=1.9x10^{-8}), which associated with variable TREC numbers. Strikingly, transplantation of human hematopoietic stem cells with the rs2204985 GG genotype into immunodeficient mice led to thymopoeisis with higher TREC levels, increased thymocyte counts and a higher TCR repertoire diversity. Our population immunology approach revealed a genetic locus that controls thymopoiesis in healthy adults, with potentially broad implications in precision medicine.

P.A2.03.04
Accelerated aging of the immune system in childhood and adolescent neuroblastoma survivors

*P. Burilova[1], K. Bendickova[1], S. S. Jose[1], T. Kepak[2,3], Z. Krenova[2], **J. Fric**[1];*
[1]Center for Translational Medicine (CTM) International Clinical Research Center (ICRC), Brno, Czech Republic, [2]Pediatric Hematology and Oncology, University Hospital Brno, Brno, Czech Republic, [3]Pediatric Oncology Translational Research (POTR), International Clinical Research Center (ICRC), St. Anne's University Hospital Brno,, Brno, Czech Republic.

Senescence of immune cells is characterized by the decline of immune functions including adaptive as well as innate responses and is associated with a number of pathologies linked to aging, including a higher susceptibility to infections or cardiovascular diseases. While senescence of adaptive immunity is relatively well described, mechanism of aging-related senescence in myeloid cells is only poorly understood. Senescence progression has been associated with several chronic inflammatory disorders but more interestingly has been observed in survivors of cancer therapy. The intensive therapeutic approach generates negative burden for patients' immune system, fueling persistent sterile chronic inflammation. By-products of cancer therapy or severe tissue damage, called damage-associated molecular patterns (DAMPs), often accumulate within the adjacent tissue and eventually spread within the blood stream. Myeloid cells and their progenitors are capable of binding DAMPs via TLRs, this results in pro-inflammatory cytokines production and initiation of inflammation. Here we address the impact of long-term administration of 13-cis-retinoic acid and topotecan currently available for neuroblastoma therapy, and DAMPs triggers to accelerated onset of immunosenescence caused by persistent low-grade inflammation. Distribution of monocytes into subsets and phagocytic activity from patient samples with neuroblastoma, the most frequent extra-cranial solid tumor in early childhood is analyzed. Furthermore, the possible impact of mentioned triggers to hematopoiesis is tested in vitro using human induced pluripotent stem cells derived myeloid cells. This project aims to develop new prognostic markers allowing to assess the progress of immunosenescence in order to prevent further serious complications, which occur long-term after the successful therapy.

P.A2.03.05
T-bet$^+$ CD8αα TCRαβ intraepithelial lymphocytesprecursor progress through a PD-1 stage and depend on C-myc

***J. Hummel**, K. Ebert, J. Fixemer, Y. Tanriver;*
University Medical Center, Freiburg i.B., Germany.

Intraepithelial lymphocytes (IELs) are a heterogeneous resident T cell population within the epithelial barrier in the small intestine and can be divided into natural (CD8αα TCRαβ or TCRγδ) and induced (CD8αβ TCRαβ) IELs. Thymic IEL precursors (IELPs) of natural CD8αα TCRαβ IELs are post-selected T cell receptor positive (TCRαβ$^+$) T cells that lack expression of the classical co-receptors CD4 and CD8 (double negative, DN) and NK1.1, which distinguishes them from natural killer T cells. We could recently refine this definition by demonstrating that lineage restriction in IELPs towards data CD8αα TCRαβ IELs is gradually imposed by the T-box transcription factor T-bet. Here, we demonstrated by employing a newly developed PD-1 fate map and conditional T-bet knockout mice that all natural IELs progress through a PD-1$^+$ stage, that is gradually lost upon T-bet expression. This transition is regulated by the transcription factor C-myc, as T cell-specific conditional C-myc knockout mice lack T-bet$^+$ IELPs, while PD-1$^+$ IELPs are still present. Using *C-myc$^{\Delta CD4}$* OT1-OVA chimera as a model of agonist-selected IELPs, we could show that high-avidity interaction cannot compensate for the loss of C-myc in IELPs. Thus, the loss of C-myc does not affect the agonistic selection of the IELP population leading to DN TCRαβ$^+$NK1.1$^-$ IELPs but lacking the final matured T-bet$^+$ IELP. Hence, the pronounced upregulation of C-myc during thymic IELP development is essential for the induction of T-bet, which in turn regulates proliferation and differentiation.

P.A2.03.06
Immunization-induced thymic atrophy as a contributing factor in strain differences in rat susceptibility to EAE

*M. Nacka-Aleksic[1], M. Stojanovic[1], I. Pilipovic[2], D. Kosec[2], **G. Leposavic**[1];*
[1]Department of Physiology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia, [2]Immunology Research Centre "Branislav Janković", Institute of Virology, Vaccines and Sera "Torlak", Serbia, Belgrade, Serbia.

Introduction: It is suggested that impaired thymopoiesis in autoimmune diseases contributes to their perpetuation. To prove this hypothesis, influence of immunization for EAE on thymopoiesis and the putative thymic-dependent changes in the periphery were examined in susceptible (Dark Agouti, DA) and resistant (Albino Oxford, AO) rats. Methods: On the 13th day post-immunization, expression of differentiation/maturation markers of conventional T cells and regulatory CD4+Foxp3+CD25+ cells (nTregs) on thymocytes, their apoptosis and proliferation, frequency of recent thymic emigrants (RTEs) and CD28^{null} cells in CD4+ and CD8+ peripheral blood lymphocytes (PBLs), and thymic expression and circulating levels of cytokines influencing thymus/thymopoiesis were investigated. Results: In rats of both strains increase in proinflammatory-cytokine circulating levels followed by thymic atrophy and changes at multiple thymocyte developmental points, leading to decreased number of the most mature CD4+ and CD8+ TCRαβ^{hi} thymocytes and frequency of RTEs among PBLs (as in chronobiological aging), was found. This was more prominent in DA rats. Consistently, compared with AO rats, in DA rats were found higher frequencies of cytolitic CD28^{null} cells (contributing to target tissue damage) among CD4+ PBLs and cytolitic granzyme B+ CD4+ T cells in spinal cord. Additionally, compared with non-immunized controls, DA rats exhibited greater decline in thymic nTreg generation (reflecting diminished thymic IL-7, IL-2 and IL-15 expression) than AO ones. Conclusions: The study suggests that differences in thymopoiesis, and consequently nTreg generation and CD4+CD28^{null} cell frequency in the periphery, contribute to strain differences in EAE clinical presentation. (Grant 175050, MESTD, Republic of Serbia).

P.A2.03.07
INF-g and TNF-α serum concentrations and frequency of IFN-g and TNF-α genotypes in children with primary immunodeficiency and recurrent respiratory tract infections (without significant immunological abnormalities)

***A. Lewandowicz-Uszyńska**[1,2], G. Pasternak[1,2], K. Bogunia-Kubik[3];*
[1]Wroclaw Medical University, Wrocław, Poland, [2]Provincial Hospital J. Gromkowski, Wrocław, Poland, [3]Polish Academy of Sciences, Wrocław, Poland.

The aim of the study was to evaluate the concentrations of INF-γ and TNF-α in children with primaryimmunodeficiency (PID). The studyincluded 93 children: 30 patients with PID, 43 RRTI, 20 healthy children (control group). The concentrations of INF-γ, TNF-α, and INF-γ and TNF-α polymorphisms were determined. The polymorphism of the TNF-alphaencodinggene (TNFA; rs1800629; -308 G / A) and IFN-gamma (IFNG; rs2430561; +874 T / A) were determined. Genotyping was done by melting curve analysis. This method is based on real-time PCR, ie PCR, where the detection of amplified DNA can be madeduring the reaction without the need for separated detection after the reaction. LightSNiPAssay (TIB MOLBIOL) and Probes Master MIX (Roche) were used for typing. Amplification was performed on LightCycler® 480 Multiwell Plate 96 plates on the LightCycler 480 II (Roche). The research was carried out in the Laboratory of Clinical Immunogenetics and Pharmacogenetics of the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences in Wroclaw. No statistically significant differences in serum INF-γ, TNF-α, serum levels were observed in the examined children. The frequency of genotypes TNFA and IFNG in the group of patients (N = 93):TNF-alpha (TNFA; rs1800629; -308 G / A) in All studiem groups was the rarest GA, the most common GG. IFN-gamma (IFNG; rs2430561; +874 T / A) in All studiem groups was the rarest genotype AA, the most common AT.

P.A2.03.09
Chronic disease development increases with suppression of acute infectious diseases in the population - a case controlled pilot study of 166 cases

***S. Mahesh**[1], M. Mallappa[1], G. Vithoulkas[2];*
[1]Centre For Classical Homeopathy, Bangalore, India, [2]University of the Aegean, Alonissos, Greece.

Introduction: The change of the world's leading cause of morbidity and mortality from infectious to non communicable diseases over the past few decades cannot be attributed to the increase in hygiene or change in lifestyle alone. This raises the question - whether the manner in which immune system morphs in response to suppression of acute inflammation is detrimental to the general health of the population. This study aimed to test the hypothesis that the development of chronic diseases increases with suppression of acute infections in the population
Materials and Methods: Teenagers opting for treatment at Centre For Classical Homeopathy, Bangalore, India were analysed for their medical history and categorised into 4 groups Group 1: Present acute infections only - recurrent or not. (control group) Group 2: Present acute infections - at least one in the past 1 year with present chronic diseases diagnosis. Group 3: Present chronic disease diagnosis with history of recurrent infections in the past. Group 4: Present chronic disease diagnosis with no history of recurrent infections in the past. Results: The trend of the chronic diseases was to cluster in group 4. The most prevalent 3 chronic conditions viz., allergic bronchitis, allergic rhinitis and chronic headaches also showed a tendency to cluster in group 4.
Conclusions: This study shows that there is a tendency for chronic diseases to be significantly higher in people without acute infections. This data may be used further to evaluate the role of different treatment modalities that suppress acute inflammation in the body.

P.A2.03.10
A network of transposable elements controls CD4 T cell fate

A. Malbec*, V. Adoue*, B. Binet*, J. Fourquet, O. Joffre;*
CPTP, Université de Toulouse, CNRS, Inserm, UPS, Toulouse, France.

CD4 T lymphocytes are highly efficient at protecting the host against endogenous and exogenous dangers. Their efficiency comes at least in part from their ability to adapt their phenotype and function to the threat detected by the cells of the innate immune system. T helper cell differentiation and commitment are strictly controlled by epigenetic mechanisms. They are necessary to establish lineage-specific gene expression programs while repressing genes associated with alternative fates. Combining genome-wide transcriptomic and epigenetic studies with functional assays, we revealed a role for a non-histone chromatin protein in CD4 T cell programming. CD4 T cells deficient for this molecule show exacerbated Th1 priming and increased Th2 cell plasticity toward the Th1 lineage. Mechanistically, we show that the deregulation of the Th1 gene expression program results from a loss of repression of a network of transposable elements. Refined bioinformatic analyses indicate that these transposons either flank and repress Th1 gene cis-regulatory elements or behave themselves as Th1 gene enhancers. In conclusion, we identified a new molecular player that ensures T cell lineage integrity by repressing a repertoire of transposable elements that have been exapted into cis-regulatory modules to shape and control the Th1 gene network.

P.A2.03.11
Beta2 integrin receptor-mediated regulation of γδ T cells

C. L. McIntyre*[1], L. Monin Aldama[2], C. S. Goodyear[1], A. Hayday[2,3], V. L. Morrison[1];*
[1]University of Glasgow, Glasgow, United Kingdom, [2]Francis Crick Institute, London, United Kingdom, [3]Kings College London, London, United Kingdom.

γδ T cells play an essential role in immune surveillance and have protective effects against several types of cancer. This has led to the development of γδ T cells as a cancer therapeutic, however the mechanisms of γδ T cell regulation and migration *in vivo* are poorly understood. αβ T cell migration and function is dependent on β_2 integrins, which are leukocyte-specific adhesion molecules, however their role in γδ T cell biology has not been fully elucidated. β_2 integrin-deficient mice develop spontaneous skin and/or oral mucosal inflammation, associated with an expansion of γδ T cells. We hypothesised that β_2 integrins are a novel regulator of γδ T cells. β_2 integrin knockout (CD18 KO) mice were used to evaluate the effect of integrin loss on γδ T cell phenotype and localisation under steady-state conditions and determine the molecular mechanism responsible for expansion. CD18 KO mice had a tissue-specific (i.e., spleen, blood, lungs, small intestine and uterus) increase in γδ T cells, suggesting the expansion is not inflammation-dependent. Interestingly, this increase in γδ T cell numbers was due to the specific expansion of $V\gamma6^+$ IL-17-producing γδ T cells. These findings are the first to highlight a potential negative regulatory role of β_2 integrins in $V\gamma6^+$ cells, indicating a new mechanism of control for this subset of γδ T cells. Further work is required to determine the mechanism of regulation, with the aim to elucidate a novel γδ T cell regulatory pathway to help inform the use of these cells in cancer therapeutics.

P.A2.03.12
Do thymic γδT cells count on the antigen receptor for effector differentiation?

S. Medrano-Garcia*[1,2], A. V. Marin[1,2], H. De La Figuera[1], J. R. Regueiro[1,2], M. Muñoz-Ruiz[3], E. Fernandez-Malavé[1,2];*
[1]Complutense University, Madrid, Spain, [2]Hospital 12 de Octubre Research Institute (imas12), Madrid, Spain, [3]The Francis Crick Institute, London, United Kingdom.

Whether the T cell receptor (TCR) is critical for the generation of effector γδ T cell subsets in the thymus is unclear. A popular view proposes that TCR signal "strength", which is normally dependent on surface TCR expression, is a major determinant of the generation of γδ T cells producing either IFN-γ or IL-17. We have recently reported that *Cd3g*$^{+/-}$ *Cd3d*$^{+/-}$ (CD3DH) mice had reduced surface TCR expression and signaling in thymic γδ T cells, and exhibited a marked depletion of IFN-γ-producing γδ T cells. Here, we have revisited the TCR signal strength model using CD3δ KO mice, whose surface TCRγδ levels are higher than in CD3DH but lower than in WT. We also analyzed CD3δ/CD3γ doubly-KO mice expressing a human CD3δ transgene (hDTg), which display consistently higher surface TCRγδ than WT. Along the developmental pathway of IFN-γ-producing γδ T cells (defined by sequential CD122 and NK1.1 expression), CD3δ KO showed a markedly increased $CD122^+NK1.1^-$ compartment and $CD122^+NK1.1^+$ cells with reduced surface NK1.1, when compared to WT. These two subsets were comparable in frequency and surface NK1.1 in hDtg and WT mice. Anti-CD3 i.p. injection reversed the apparent blockade at the $CD122^+NK1.1^-$ stage of CD3δ KO. On the other hand, the relative abundance of putative IL-17-commited γδ T cells ($CD27^-CCR6^+$) was reduced in CD3δ KO but augmented in hDTg mice, particularly in a unique population displaying the highest TCR levels. Our study supports the existence of distinct TCR expression/signaling requirements for effector γδ T cell differentiation in the thymus.

P.A2.03.13
Dielectric properties of serum in children with recurrent respiratory tract infections

G. Pasternak*[1,2], D. Łuczycka[3], K. Pentoś[3], K. Gul[1,2], M. Kaźmierowska-Niemczuk[1], A. Lewandowicz-Uszyńska[1,2];*
[1]Wroclaw Medical University, Wrocław, Poland, [2]Provincial Hospital J. Gromkowski, Wrocław, Poland, [3]Wroclaw University of Environmental and Life Sciences, Wrocław, Poland.

Despite the improvement of living conditions, availability of medicines and various methods of treatment, recurrent respiratory tract infections continue to occur and are a frequent clinical problem of children of our climate zone (moderate warm intermediate nature). It results, among others, from the geo-climatic conditions, lifestyle, and maturation of the immune system (especially in humoral immunity) in developmental age. One of the reasons for these ailments is a variety of abnormalities in the immune system that may have of primary or secondary nature. We are still looking for new conditions that may underlie recurrent infections. Various screening assays are also being tested to demonstrate quickly and easily abnormalities in selected parameters of the immune system, so that detailed, cost-intensive and time-consuming immunoassays can be performed in justified cases.
The aim of the study was to examine the occurrence of dependence between selected physical parameters of serum such as: electrical conductivity, electrical permeability, dielectric loss factor, and blood tests results in the following: blood counts, serum glucose concentration, micronutrient concentrations, and selected parameters of the immune system: concentrations of the main classes of immunoglobulins (IgG, IgA, IgM and IgE), complement hemolytic activity, and neutrophil function in patients suffering from recurrent respiratory tract infections compared to those without recurrent infections.
During the impedance spectroscopy of the tested samples, there was a differentiation of the obtained measurement results. In order to develop the obtained measurement and analytical data, a correlation matrix was determined and a grouping of the analyzed cases was carried out.

P.A2.03.14
Phenotypic characterization of immune cells from premature neonates with extreme low birth weight

K. Qazi Rahman*;*
Stockholm University, Stockholm, Sweden.

Background: The development of the immune system begins during the first trimester, but continues to develop after birth. Premature children have a compromised immune system with regard to both quantitative and qualitative aspects. There is an increased risk of sepsis, necrotizing enetrocolitis and pneumonia-all common causes of mortality, primarily in the group of neonates with extremely low birth weight (ELBW).
Objective: To characterize innate and adaptive immune compartments of ELBW infants 14 days after birth.
Materials and methods: Peripheral blood mononuclear cells (PBMC) were collected 14 days after birth from 79 ELBW premature infants, participating in a randomized double-blind placebo-controlled study of probiotic supplementation. As a control, PBMCs from 29 full-term (FT) infants at 14 days of age were used. Mononuclear cell populations were analyzed by multi-colour flow cytometry.
Results: The proportions of CD4 and CD8 T cells, regulatory T cells, NK cells and monocytes were significantly lower in PBMC of ELBW premature infants compared to FT infants. On the contrary, γδ T cell- and NKT cell frequencies were comparatively high in the ELBW infants. Further, an elevated CD4:CD8 ratio was observed in ELBW premature infants. The expression levels of homing receptors CCR4 and CCR9 on T cells were high, while monocyte expression of HLA-DR and CD86 was very low in ELBW infants.
Conclusion: We provide an extensive characterization of the peripheral immune compartment of ELBW premature infants that provides important information for future studies on immune function and possible therapeutic interventions.

P.A2.03.15

Infant Immune Responses following Pertussis Vaccination in Pregnancy

T. Rice[1], *D. Diavatopoulos*[2], *B. Donaldson*[1], *M. Bouqueau*[1], *H. Sallah*[1], *B. Kampmann*[1,3], *B. Holder*[1];
[1]*Imperial College London, London, United Kingdom,* [2]*Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands,* [3]*Vaccinology Theme, MRC Unit, Gambia.*

Introduction: Following a surge in pertussis cases in the UK disproportionately affecting young infants, a maternal vaccination program was introduced. We established a longitudinal study to investigate the impact of maternal Tdap (tetanus, diphtheria, acellular pertussis) immunisation on the developing infant immune system, including antibody production and cellular responses to *Bordetella pertussis* following vaccination of both mothers and infants.
Methods: Blood was collected from Tdap-vaccinated/unvaccinated pregnancies: maternal at booking (8-21weeks), maternal and cord at birth, and infants at 7weeks and 5months (pre- and post- their primary immunisations). Antibody levels directed against pertussis vaccine antigens (Prn, FHA, PTx, DTx, TTx) were measured using a fluorescent bead-based multiplex immunoassay. Cellular responses to *Bordetella pertussis* were measured by whole blood stimulations with strains of heat-killed pertussis (wild-type, Prn-negative, Prn/FHA-negative) followed by multiplex cytokine analysis.
Results: Maternal Tdap vaccination significantly increases vaccine-specific antibody titres in women infants before primary immunisation. Post-childhood vaccination antibody titres were the same between babies born to vaccinated and unvaccinated pregnancies by 5 months of age. Cytokine responses to *B.pertussis* in vaccinated and unvaccinated groups were strain-dependent, with several cytokines elevated in response to the Prn/FHA-negative strain. Age-dependent cytokine responses were seen, that may be altered in infants from vaccinated pregnancies.
Conclusion: Maternal pertussis vaccination boosts protective antibody prior to the first dose of infant vaccination.
There was no impact on infants' antibody response to DTaP vaccination. Like adults, infants mount strain-dependent cytokine responses to *B.pertussis* that are not impacted by maternal vaccination. Age-dependent effects are under investigation.

P.A2.03.16

Age-related defects in efferocytosis are reversed by inhibiting p38 MAPK activity in aged volunteers

R. C. van de Merwe, *R. P. De Maeyer, A. N. Akbar, D. W. Gilroy;*
University College London, London, United Kingdom.

Ageing is associated with chronic inflammation, which may be due to failed resolution. As p38 MAPK regulates pro-resolution pathways including efferocytosis, we investigated p38 MAPK signaling in aged versus young humans using a skin blister model of self-limiting acute inflammation. Thereafter, we used the p38 MAPK inhibitor losmapimod to discern p38 MAPK's role in the onset and resolution of inflammation in the aged. Cantharidin (0.1%) was applied topically to young (<40 years) and aged (>65 years) human volunteers. A cohort of aged volunteers were exposed to losmapimod (15mg BID/PO) for 4d prior to cantharidin application. Blister exudates were collected at 24+72h and were analysed by flow cytometry. Phospho-p38 MAPK was increased in HLA-DR+ blister cells in aged compared to young volunteers. In aged volunteers (-losmapimod) early apoptotic cells accumulated at the 72h resolution time point; a finding not observed in young volunteers. Furthermore, PMN clearance in aged volunteers (-losmapimod) did not correlate with $CD14^{hi}$ mononuclear phagocyte (MPs) numbers in contrast to young volunteers. This indicated failed apoptotic body clearance in the aged, which we hypothesise arises from defective efferocytosis. Aged MPs expressed lower TIM-4, a molecule involved in apoptotic body clearance, compared to younger counterparts. In aged volunteers losmapimod elevated TIM-4 expression on MPs, increased MP numbers at resolution, and rescued the correlation between MP numbers and PMN clearance. Inhibition of p38 MAPK in aged humans alters the cellular profile of cantharidin blisters, and may enhance efferocytosis during the resolution of inflammation by increasing TIM-4 expression on MPs.

P.A2.03.17

Homeostatic cytokines revert primed $CD8^+$ T cells to naïve-like memory stem cell variants through epigenetic and transcriptional reprogramming

G. Frumento[1,2], ***K. Verma***[1], *W. Croft*[1,3], *A. White*[1], *Z. Nagy*[4], *S. Kissane*[5], *S. P. Lee*[1], *G. Anderson*[1], *P. Moss*[1,6], *F. E. Chen*[1,2,6];
[1]*Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, United Kingdom,* [2]*NHS Blood and Transplant, Birmingham, United Kingdom,* [3]*Centre for Computational Biology, University of Birmingham, Birmingham, United Kingdom,* [4]*Institute of Inflammation and Ageing, Univeristy of Birmingham, Birmingham, United Kingdom,* [5]*Technology Hub, University of Birmingham, Birmingham, United Kingdom,* [6]*Centre for Clinical Haematology, University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom.*

INTRODUCTION: Upon activation, human naïve T-cells are believed to differentiate into memory cell subsets along a one-way pathway, acquiring effector function but diminishing proliferative capacity and survival. Two subsets of phenotypically naïve $CD8^+$ T-cells (T_{SCM} and T_{MNP}) with stem cell features, memory traits and enhanced proliferative potential have also been described although there is little understanding on how they are generated. Here we analyzed the epigenetic and transcriptional regulation of T-cell differentiation/de-differentiation upon stimulation with homeostatic cytokines.
METHODS: CD8+ T_N cells were isolated from cord or peripheral blood for *in-vitro* stimulation with homeostatic cytokines. Cells were sorted on the basis of membrane expression of CD45RA and CCR7 for analysis of open chromatin by ATAC-seq, for transcriptional analysis by microarray and for phenotypic and functional assays.
RESULTS: We demonstrate that Interleukin-7 can de-differentiate recently-differentiated memory $CD8^+$T cells into revertant T_N-like cells (T_{Nrev}). We show that these T_{Nrev} share phenotypic and functional characteristics with T_{SCM} and T_{MNP} including high proliferative and differentiation capacity and polyfunctionality when re-stimulated. ATAC-seq indicates that T_{Nrev} fit between recently differentiated central memory and effector memory T-cells. Phenotypic reversion is seen to be driven by Notch signaling pathway, ETS family transcription factors, Stat1 and Stat4.
CONCLUSION: Our study provides a novel model for $CD8^+$ T_N differentiation and a unifying theory for the generation of T_{SCM} and T_{MNP}. We suggest that cytokine-dependent reversion of recently-differentiated $CD8^+$ T-cells replenishes the early-memory T-cell pool for preserving long-term immunity. This mechanism can be used to generate *in-vitro* engineered early-memory T-cells for immunotherapy.

P.A2.03.18

Human milk-derived extracellular vesicles can modulate epithelial and immune cell responses

M. I. Zonneveld[1,2], *M. J. van Herwijnen*[1], *M. M. Fernandez-Gutierrez*[3], *A. de Groot*[4], *M. Kleinjan*[1], *T. M. van Capel*[5], *A. J. Sijts*[4], *L. S. Taams*[6], *J. Garssen*[2,7], *E. C. de Jong*[5], *M. Kleerebezem*[3], *E. N. Nolte-'t Hoen*[1], *F. Redegeld*[2], ***M. H. M. Wauben***[1];
[1]*Utrecht University, Faculty of Veterinary Medicine, Department of Biochemistry & Cell Biology, Utrecht, Netherlands,* [2]*Utrecht University, Faculty of Science, Department of Pharmaceutical Sciences, Utrecht, Netherlands,* [3]*Wageningen University, Department of Animal Sciences, Host-Microbe Interactomics Group, Wageningen, Netherlands,* [4]*Utrecht University, Faculty of Veterinary Medicine, Department of Infectious Diseases and Immunology, Division Immunology, Utrecht, Netherlands,* [5]*University of Amsterdam, AMC, Department of Experimental Immunology, Amsterdam, Netherlands,* [6]*King's College London, School of Immunology and Microbial Sciences, Department Inflammation Biology, London, United Kingdom,* [7]*Nutricia Research, Utrecht, Netherlands.*

Breast milk is nature's first functional food with distinctive functions: it provides nutrition and it supports the development of the gastrointestinal (GI) tract and the immune system. Remarkably, little is known about the precise constituents in breast milk responsible for these effects. Previous research focused on the identification of individual functional milk components while the functionality of macromolecular components in milk remained largely understudied. Extracellular vesicles (EV), submicron lipid bilayer enclosed vesicles released by cells for intercellular communication, belong to these macromolecular milk components. Recently, we unveiled a novel functional milk proteome associated to milk-EVs. Next, we analyzed functional effects of milk EVs in various in vitro assays and found that physiological concentrations of milk EV support epithelial barrier function, by increasing epithelial cell migration via the p38 MAP kinase pathway. Furthermore, milk EV inhibited agonist-induced activation of TLR3, 7 and 9 and inhibited activation of CD4+ T cells by temporarily suppressing T cell activation without inducing tolerance or suppressive regulatory T cells. Integrative analysis of these data with our milk EV-proteome data indicated that EVs contain multiple proteins that can modulate signaling pathways involved in migration, TLR signaling and T cell activation at various levels. Our results demonstrate that human milk EV are multi-signaling vehicles that can selectively control the activation and inhibition of various signaling pathways that are crucial for immune homeostasis and the development of the infant GI tract. (This study was performed within a partnership program by Nutricia Research and the Dutch Technology Foundation STW (11676)).

P.A2.03.19

Aggravated aging-related immune changes areassociated with inflammation and cardiovasculardisease in end-stage renal disease patients: baselinefindings from the iESRD study

***K. Shu**, Y. Chiu;*
Far Eastern Memorial Hospital, New Taipei City, Taiwan.

Patients with end-stage renal disease (ESRD) exhibit accelerated aging of the immune system and increased risk for cardiovascular disease, but the etiology and overall contribution of immune system aging, or immunosenescence, to cardiovascular disease is not well understood. We performed a comprehensive lymphocyte and monocyte immunophenotyping in 412 ESRD patients on maintenance hemodialysis and 57 age-matched healthy individuals. Compared with healthy individuals, ESRD patients had decreased levels of naive CD4+ and CD8+ T cells and increased levels of terminally differentiated (CCR7-CD45RA+) T EMRA cells and intermediate monocytes (CD14++CD16+). These changes not only were significantly correlated with age but also were enhanced by longer dialysis vintage. Lymphocyte and monocyte aging also correlated with other established cardiovascular risk factors, including hemoglobin and high-sensitivity C- reactive protein. In multivariable-adjusted logistic regression models, the combination of high terminally differentiated CD8+ T EMRA cell level and high intermediate monocyte levels, as a predictive immunophenotype, was independently associated with the existence of coronary artery disease (OR=2.29, 95% CI=1.2~4.5, p=0.016) as well as cardiovascular disease including stroke and peripheral arterial occlusive disease (OR=2.32, 95% CI=1.2~4.4, p=0.008). We also found evidence that terminal differentiated T cells were enhanced by the uremic toxin indoxyl sulfate. Our work indicates that cardiovascular disease in the ESRD population might be enhanced by the presence of accelerated aging-associated immune changes consequent to long-term exposure to uremic toxins.

P.A2.03.20

The source of IL-1β expression and its role in type 2 diabetes mouse models

***J. Wehner**, S. Wiedemann, D. T. Meier, S. P. Häuselmann, M. Böni-Schnetzler, M. Y. Donath;*
University of Basel, Basel, Switzerland.

Type 2 diabetes is an inflammatory disease and a worldwide problem. A critical aspect of type 2 diabetes is insulin resistance and the impairment of beta cell function in which cytokines play a key role. Cytokines, in particular IL-1β, mediate chronic low-grade inflammation in pancreatic islets. However, the primary source of IL-1β in islets is unclear.
We hypothesize β-cells or macrophages as potential producers of IL-1β. While β-cells are the main cell type in islets, it is unknown if they are able to process pro-IL-1β to IL-1β. Macrophages are known as potent producers of IL-1β, but rare in numbers in pancreatic islets. Pro IL-1β overexpression could reveal the processing and causal effect of IL-1β from different sources.
We use two strains of mice in our study:
- Inducible β-cell specific pro-IL-1β overexpressing
- Constitutive myeloid cell specific pro-IL-1β overexpressing
We investigated resulting phenotypes in vivo and in vitro.

P.A2.03.21

Proliferation of T-regulatory cells and expression by them of CTLA-4 under the influence of humoral factors of homeostatic proliferation in healthy donors.

***D. Shevyrev**, E. Blinova, E. Pashkina, L. Grishina, V. Kozlov;*
Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation.

Homeostatic proliferation (HP) is the main mechanism of T-cells pool reconstitution in adulthood. It is a well-known fact about the link between HP and disturbance of peripheral tolerance, which normally provided by T-regulatory cells (Treg). However it remains unclear why these cells can't prevent autoimmunity under HP. The purpose of this study is to improve our knowledge about effects of IL-7 and IL-15 - the main humoral factors of HP on T-regulatory cells.The study included 6 healthy donors. Treg-cells proliferation and ones expression of CTLA-4 were analyzed under HP cytokines (50ng/ml for IL-7 and IL-15) and antiCD3+IL-2 (1mkg/ml and 100ME/ml respectively) during 7-day cultivation with PBMC in ratio 1:1. Immune-magnetic separation was used for Treg-cell isolation (purity>95%). Phenotyping of cells was performed by flow cytometry.The research revealed, that IL-7 and IL-15 can effectively maintain Treg-cells by number and phenotype, but Treg-cell proliferation was significantly lower than in $CD4^+$T-lymphocytes under HP factors. In addition, it was found considerable decline of CTLA-4 surface expression on Treg-cells unlike for $CD8^+$T-lymphocytes under HP cytokines ($p<0,05$).We identified that proliferation of Treg-cells under HP factors is much lower than in $CD4^+$T-lymphocytes, which can lead to a delay of Treg-pool reconstitution under lymphopenia and may represent another mechanism of link between HP and autoimmune disorders. In addition, we revealed decrease of CTLA-4 expression - one on Treg-cells under influence of HP cytokines that also can contribute disruption of self-tolerance and development of autoimmunity. This study was funded by RFBR and Novosibirsk region, project №17-44-540167.

P.A2.03.22

Long term immune dysfunction induced by sepsis is dependent of age

***D. F. Colon**[1], C. Wanderley[2], A. L. Souza[1], F. Castanheira[3], P. Donate[1], A. P. Carlotti[1], F. Carmona[1], F. Ramalho[1], J. C. Alves-Filho[1], F. Y. Liew[4], F. Q. Cunha[1];*
[1]Universidade de São Paulo, Ribeirão Preto, Brazil, [2]Universidade Federal de Ceará, Fortaleza, Brazil, [3]University of Calgary, Calgary, Canada, [4]University of Glasgow, Glasgow, United Kingdom.

Introduction: Patients who survive sepsis can develop long-term immune dysfunction, with expansion of M2 macrophages and regulatory T cells. However, there is no evidence of these alterations in the pediatric sepsis. Aim: To investigate the role of age in the genesis of immunosuppression following sepsis. Methods: infant and adult mice were submitted to sepsis and treated with antibiotic. On day 15 after infection, Treg cells frequency and the activation of IL-33/Th2 cytokines/ILC2/M2 macrophages axis were performed. Furthermore, surviving sepsis mice were inoculated intranasally with *Pseudomonas aeruginosa* or injected subcutaneously with B16LucF10 cell line. Moreover, blood samples from sepsis-surviving patients were collected and the Treg cells and Th2 cytokines were evaluated.
Results: Here we showed that sepsis surviving-infant mice, in contrast to adults, were resistant to secondary infection and controlled the tumoral growth suggesting the non-development of immunosuppression.
Mechanistically, infant group exhibited a decrease in *Foxp3* expression, lower phosphorylation of SMAD2/3 and reduction in Tregs cell expansion and FOXP3 stability. Furthermore, infant mice presented lower IL-33 and Th2 profile cytokines (IL-4 and IL10) production as well as lower expansion of ILC2 cells, leading to progressive decrease in the M2 macrophages and Tregs cell expansion. Importantly, sepsis-surviving pediatric patients, in contrast to adults, did not exhibit increase in Treg cell, IL-33 and IL-10 in their peripheral blood. Conclusion: These findings demonstrate for the first time that the sepsis immunosuppression is related to the age. Thus, a better understanding of the process could lead to differential therapeutic treatments of adult and pediatric sepsis.

P.A2.03.23

Transcriptional and functional landscape of HERV-K (HML-2) in aging

***A. Autio**[1], T. Nevalainen[1,2], B. Mishra[1], M. Hurme[1,2];*
[1]Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland, [2]Gerontology Research Center (GEREC), University of Tampere, Tampere, Finland.

An estimated 8% of the human genome consists of endogenous retroviruses (HERV), which are genetic remnants from past retroviral infections. Millions of years' worth of mutational decay has rendered most HERV proviruses inactive, yet some still contain intact reading frames and can even code for functional products. Upregulation of these retroelements has been observed in aging and senescent cells.
To study the aging-associated changes in the intensity of transcriptional activation of recently integrated HERV-K (HML-2) subfamily, we utilized RNA-sequencing of PBMCs obtained from elderly cases (n=7, age 90) and young controls (n=7, age 26-32, median age 28). The correlations in expression between endogenous genes and proviruses were calculated and gene set enrichment analysis (GSEA) was done. The known gene ontology (GO) terms of genes may indicate biological processes that the similarly expressed proviruses are involved in.
We found that a third (33/91) of the HERV-K (HML-2) proviruses were relatively strongly expressed (read count >=16). The general level of expression was similar across age groups, yet hierarchical clustering of samples indicated aging-associated differences in expression patterns. Three proviruses were significantly differentially expressed. The proviral expression in the old individuals was associated with a greater number of biological processes (GO terms).
Our study of the expression of HERV-K (HML-2) proviruses in old and young individuals has provided candidate biological processes that may be involved in the interplay of genes, proviruses, and aging. Further investigation of these processes could uncover potential links between proviruses and aging-associated changes, such as immunosenescence and inflammaging.

P.A2.04 Immune development and aging from the cradle to the grave - Part 4

P.A2.04.01
Cereblon regulates hematopoietic stem cell self-renewal

A. A. Akuffo[1,2], *J. M. Billington*[1], *D. E. Muench*[3], *J. L. Cleveland*[1], *H. L. Grimes*[3], *P. K. Epling-Burnette*[1];
[1]*H. Lee Moffitt Cancer Center, Tampa, United States,* [2]*University of South Florida, Tampa, United States,* [3]*Cincinnati Children's Hospital Medical Center, Cincinnati, United States.*

Introduction: Cereblon (CRBN), an E3 ubiquitin ligase substrate receptor and target of immunomodulatory drugs, is ubiquitously expressed in hematopoietic lineages, including hematopoietic stem cells (HSCs) [lineage$^-$Sca$^-$1$^+$c-Kit$^+$SLAM$^+$(LSKs)]. Immunomodulatory drugs restore erythropoiesis and suppress malignant clonal stem-cell expansion in chromosome 5q-deleted myelodysplastic syndrome, suggesting that CRBN may regulate hematopoiesis.
Materials and Methods: HSCs, progenitors, and mature populations were assessed in the bone marrow (BM) and peripheral blood (PB) of *Crbn* deficient mice (*Crbn*$^{-/-}$) and wild-type littermates (*Crbn*$^{+/+}$). To assess if *Crbn* loss induces functional differences in HSC, we performed chimeric bone marrow (BM) transplants, short-term *in vitro* colony formation and forced LSK self-renewal *in vivo* with 5-fluorouracil (5-FU) treatment. RNA sequencing of LSKs was used to identify targets controlled by CRBN. Pimonidazole staining (active in <1% O_2) was used to assess LSK localization within the endosteal niche.
Results: *Crbn*$^{-/-}$ mice have increased numbers of mature and committed myeloid populations in the peripheral blood and BM in association with increased colony numbers and increased mature myeloid populations following chimeric HSC transplants. Notably, however, total LSKs and LSK subpopulations in the BM are reduced 3-5-fold in *Crbn*$^{-/-}$ mice, and this is accompanied by lethal BM failure following repeated 5-FU dosing. Interestingly, RNA-seq analysis revealed markedly reduced expression of the hypoxia-inducible factor HIF1α in *Crbn*$^{-/-}$ LSK and lower pimonidazole staining by LSKs.
Conclusions: Collectively, these findings suggest that loss of CRBN impairs endosteal niche localization of LSK, which triggers HSC proliferation and premature exhaustion.

P.A2.04.02
Traf2 and Nck-interacting Kinase Is a Conserved Regulator of Self-Renewal and Quiescence in Hematopoietic and Leukemia Stem Cells

M. A. Amrein[1], *E. D. Bührer*[1], *S. Höpner*[1], *C. Jaeger*[1], *C. Riether*[1,2], *A. F. Ochsenbein*[2];
[1]*Department of BioMedical Research, University of Bern, Bern, Switzerland,* [2]*Department of Medical Oncology, Inselspital, Bern University Hospital, Bern, Switzerland.*

The Traf2 and Nck-interacting kinase (TNIK) is a member of the germinal center kinase (GCK) family. TNIK is known effector kinase of TRAF6, TRAF2 and beta-catenin and is involved in the integration of NFk-B, JNK and Wnt-Signaling. We found *Tnik* to be widely expressed in hematopoietic cells, including hematopoietic stem cells (HSCs). To explore how TNIK signaling regulates HSCs and leukemia stem cells (LSCs), we generated a constitutive *Tnik*$^{-/-}$ mouse. Under steady-state conditions Tnik deficiency resulted solely in a decreased frequency of quiescent long term HSCs, while the *in vitro* self-renewal capacity and the composition of bone marrow (BM), blood and spleen remained unchanged. Next we tested the responsiveness of *Tnik*$^{-/-}$ HSCs in response to hematopoietic injury and stress. Recovery after injection of 5-fluorouracil was significantly improved in *Tnik*$^{-/-}$ mice compared to littermate controls. Moreover, the reconstitution potential in competitive BM chimeras was significantly improved in *Tnik*$^{-/-}$ HSCs compared to competitor *Tnik*$^{wt/wt}$ HSCs. Interestingly, in a murine model of chronic myeloid leukemia (CML) Tnik deficiency severely impaired the self-renewal capacity of LSCs *in vitro* and *in vivo*. To summarize, we have described for the first time a role for Tnik in the regulation of quiescence and self-renewal in HSCs and CML LSCs. Overall our data suggests that TNIK might be an attractive therapeutic target to improve hematopoietic reconstitution or to eliminate CML LSCs. The exact mechanisms by which TNIK regulates self-renewal and quiescence in HSCs and LSCs remains to be studied.

P.A2.04.03
MAZR controls the development and differentiation of Foxp3$^+$ regulatory T cells

L. Andersen[1], *A. Guelich*[1], *M. Alteneder*[1], *T. Preglej*[1], *N. Dhele*[1], *A. Hladik*[2], *S. Knapp*[2], *S. Floess*[3], *J. Huehn*[3], *T. Krausgruber*[4], *C. Bock*[4], *T. Faux*[5], *A. Laiho*[5], *L. Elo*[5], *O. Rasool*[6], *R. Lahesmaa*[6], *T. Sparwasser*[7], *S. Sakaguchi*[1], *W. Ellmeier*[1];
[1]*Institute of Immunology, Medical University of Vienna, Vienna, Austria,* [2]*Laboratory of Infection Biology, Department of Medicine I, Medical University, Vienna, Austria,* [3]*Experimental Immunology, Helmholtz Centre for Infection Research, Braunschweig, Germany,* [4]*Research Center for Molecular Medicine, CeMM, Vienna, Austria,* [5]*Computational Biomedicine Lab, Turku Centre for Biotechnology, Turku, Finland,* [6]*Molecular Systems Immunology, Turku Centre for Biotechnology, Turku, Finland,* [7]*Institute of Infection Immunology, Twincore, Hannover, Germany.*

Foxp3$^+$ regulatory T cells (T_{reg} cells) play a key role in maintaining immune homeostasis and in modulating immune reactions during infection and disease. Hence it is important to understand the molecular mechanisms that regulate their development. Here we report that the BTB zinc finger transcription factor MAZR (also known as Patz1) crucially controls Foxp3$^+$ regulatory T cell generation and differentiation. We detected that MAZR was highly expressed in the thymus and gradually downregulated during progressive T cell development. Interestingly, lowest MAZR expression levels were found in terminally differentiated Foxp3$^+$ T_{reg} cells. By comprehensively analyzing mice with a T cell-specific deletion (*Cd4*-Cre) of MAZR (MAZR-cKO) we observed that the deletion of MAZR led to a substantial increase in Foxp3$^+$ Treg cells *in vivo*. Moreover, naïve MAZR-null CD4$^+$ T cells differentiated with an enhanced frequency into *in vitro*-generated iT_{reg} cells, while retroviral-mediated enforced expression of MAZR impaired the generation of T_{reg} cells both *in vitro* and *in vivo*. In DSS-induced colitis, MAZR-cKO mice were better protected against disease than their WT littermates, and *ex vivo*-isolated MAZR-null T_{reg} cells displayed slightly enhanced suppressive activity compared to WT cells. However, in an adoptive T cell transfer colitis model, MAZR-null T_{reg} cells did not display an enhanced protective activity, which also correlates with RNA-seq data revealing only minor transcriptional changes in MAZR-null T_{reg} cells. Together, our data strongly suggest that MAZR is a negative regulator of Foxp3$^+$ T_{reg} cell development and differentiation. Support by: FWF-Projects P23641,P26193,P29790; and by Horizon-2020 Marie-Sklodowska-Curie grant (No:675395).

P.A2.04.04
Characterization of the developmental landscape of iNKT17 cells

s. li[1,2], *d. al dulaimi*[1,2], *j. klibi*[1,2], *c. joseph*[1,2], *s. brunet*[1,2], *m. delord*[1,3], *v. parietti*[1,4], *j. jaubert*[1,5], *j. marie*[1,6], *s. karray*[1,2], *g. eberl*[1,7], *b. lucas*[1,8], *a. toubert*[1,2], ***k. benlagha***[1,2];
[1]*inserm, paris, France,* [2]*1INSERM, UMR-1160, Institut Universitaire d'Hématologie, Paris, France ; Université Paris Diderot, Sorbonne Paris Cité, Paris, France., Paris cedex 10, France,* [3]*2Plateforme de Bioinformatique et Biostatistique, Institut Universitaire d'Hématologie, Université Paris Diderot, Sorbonne Paris Cité., Paris cedex 10, France,* [4]*3Département d'Expérimentation Animale, Institut Universitaire d'Hématologie, Paris, France ; Université Paris Diderot, Sorbonne Paris Cité., Paris cedex 10, France,* [5]*4Mouse Genetics Unit, Institut Pasteur, Paris, France., Paris, France,* [6]*5Department of Immunology, Virology and Inflammation, Cancer Research Center of Lyon UMR INSERM1052, CNRS 5286, Centre Léon Bérard Hospital, Université de Lyon, Equipe labellisée LIGUE, Lyon, France., Lyon, France,* [7]*6Microenvironment &Immunity Unit, Institut Pasteur, Paris, France, and INSERM U1224, Paris, France., Paris, France,* [8]*7Institut Cochin, Centre National de la Recherche Scientifique UMR8104, INSERM U1016, Université Paris Descartes, 75014 Paris, France, Paris, France.*

Invariant natural killer T cells (iNKT) expressing RORγt represent a minor subset of CD1d-restricted iNKT cells with phenotypic and functional features related to Th17 cells. In this study, we aimed to understand RORγt$^+$ iNKT17 cells distribution and the sequence of events accompanying their thymic development. We found that early HSA^{high} RORγt$^+$ iNKT cell precursor are not exclusive to the iNKT17 lineage, and show that mature HSA^{low} iNKT17 cells develop through CCR6$^-$CD138$^-$ stage 1', CCR6$^-$CD138$^+$ stage 2', and CCR6$^+$CD138$^+$ final stage 3'. Stage 1' iNKT17 cells do not produce IL-17 and have the propensity to apoptosis. They will progressively express RORγt, acquire the capacity to produce IL-17, cease division, and became less prone to apoptosis. iNKT17 cells do not reside in the thymus contributing, in addition to apoptosis, to their minor partition in the thymus. Finally, we found that iNKT17 cells perceive a strong TCR signal that could promote their entry into a specific "Th17 like" developmental program impacting their survival and emigration. Overall, our studies unravel a thymic developmental sequence for iNKT17 cells, which can be of great use to study molecular mechanisms regulating this developmental program.

P.A2.04.05
Heterogeneity of CD8$^+$ terminally differentiated effector T-cells (TEMRA): chemotactic features and effector potential of different subsets

L. Burkhardt[1,2], *I. Kotko*[1,2], *S. Schlickeiser*[1,2], *D. Kunkel*[2], *A. Jurisch*[1], *C. Giannini*[1,2], *B. Sumpf*[1,2], *S. Reinke*[2], *M. Streitz*[1], *D. Wendering*[1,2], *M. Schmück-Henneresse*[1,2], *H. Volk*[1,2];
[1]*Institute for Medical Immunology, Berlin, Germany,* [2]*Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Berlin, Germany.*

Terminally differentiated effector memory CD8$^+$ T-cells (TEMRA) are triggered by chronic antigen exposition and can either be defined conventionally as CD3$^+$CD8$^+$CCR7$^-$CD45RA$^+$ T-cells or as CD3$^+$CD8$^+$CD57$^+$CD28$^-$ T-cells. TEMRA are ambiguously described as highly pre-activated or rather 'senescent' T-cells. Moreover, they show beneficial features during chronic viral infections but also worst effects on tissue regeneration.
We hypothesized, that these opposing findings implicate the phenotypic and functional heterogeneity of CD8$^+$ TEMRA cells.
Previous phenotyping by multiplex mass cytometry (CyTOF) affirmed the presumed heterogeneity within the TEMRA population. Thus, we could define four major subpopulations within TEMRA (CCR7$^-$CD45RA$^+$) using CD57 and CD28. Further, we discovered a specific subset expressing CD182 (IL8R/CXCR2) within the CD57$^+$CD28$^-$ TEMRA. This receptor is shown to trigger chemotaxis of the CD57$^+$CD28$^-$CXCR1$^+$CXCR2$^+$ TEMRA towards IL8 and seems to be required for the accumulation of this unique subset at sites of sterile intratissue inflammation. This goes in line with our data on the accumulation of CD8$^+$CD57$^+$CD28$^-$ TEMRA in fracture hematomas causing a delayed healing. Additionally, this subset showed strong cytotoxic potential, indicated by the high expression of CD107a after PMA/Ionomycin stimulation. Furthermore, a distinct subset of CD28$^+$CXCR1$^-$CXC2$^-$CXCR3$^+$CCR5$^+$ TEMRA migrated towards CXCL9, CXCL10 and CCL3. Interestingly, the inflammatory potential (TNFα/IFNγ/IL2) of TEMRA subsets increased with the expression of the CD28 molecule. Whereas, CD28$^-$ subsets showed the lowest inflammatory capacity, they required less stimulatory signals and are more resistant to negative checkpoint signals. The data supports the differential impact of TEMRA subpopulations on inflammation/immunity dependent on the respective chemokine receptor expression and inflammatory/cytotoxic potency.

P.A2.04.06

Developmental origins of distinct adipose tissue macrophages in health and disease

***Q. Chen**, R. Christiane;*
School of Biological Sciences, Singapore, Singapore.

Adipose tissue macrophages (ATMs) are the main leukocytes found in the visceral adipose tissue (VAT) which play an important role not only in an effective cell debris clearance, but also in controlling tissue immune surveillance as well as lipid buffering.
Because of the prenatal ontogeny of many tissue-resident F4/80^{hi} macrophages, such as in the liver and lungs, we addressed the question of the origin and turnover kinetics of distinct subpopulations of ATMs in young, aged and obese mice. To determine whether ATMs are derived from adult bone marrow (BM) or from embryonic haematopoiesis, we exploited a *Kit*$^{MerCreMer/R26}$ fate mapping mouse - where YFP expression can be induced by tamoxifen injections in early BM progenitors - to monitor the turnover rates driven by the BM input in distinct VAT myeloid cell populations. In lean young mice, our fate map analysis revealed the presence of two CCR2-independent F4/80^{hi} populations with slow turnover kinetics when compared to conventional monocyte-derived CCR2^{+} macrophages which are rapidly refilled by BM-derived monocytes. During aging and in particular in obesity, a third F4/80^{hi} ATM population expressing high levels of CD11c infiltrates the VAT showing a fast turnover dynamics and a clear BM-dependency. Taken together, our data identifies distinct ATMs subpopulations in the VAT which in normal healthy conditions can maintain them self almost independently from any BM input, but under inflammatory conditions, such as obesity, are rapidly replenished by BM-derived monocytes.

P.A2.04.07

Influence of influenza A virus infection on thymic development of Foxp3^{+} regulatory T cells

***Y. Elfaki**[1], M. Gereke[2,3], N. Tafrishi[4,5], I. Schmitz[4,5], D. Bruder[2,3], S. Floess[1], J. Huehn[1];*
[1]Helmholtz Centre for Infection Research, Experimental Immunology, Braunschweig, Germany, [2]Helmholtz Centre for Infection Research, Immune Regulation, Braunschweig, Germany, [3]Otto-von-Guericke University, Institute of Medical Microbiology, Infection Control and Prevention, Magdeburg, Germany, [4]Helmholtz Centre for Infection Research, System-oriented Immunology and Inflammation Research, Braunschweig, Germany, [5]Otto-von-Guericke-University, Institute for Molecular and Clinical Immunology, Magdeburg, Germany.

Foxp3^{+} regulatory T cells (Tregs) are crucial for maintenance of self-tolerance and regulation of inflammatory responses against pathogens. The majority of Tregs develops within the thymus, termed thymus-derived Tregs (tTregs). Since influenza A virus (IAV) infection has been reported to cause transient thymic involution, it might potentially impact tTreg development and thereby have long-lasting consequences for immune homeostasis and self-tolerance. Thus, this project aims at dissecting the influence of IAV infection on tTreg development. To this end, thymi of IAV-infected Foxp3^{hCD2}×RAG^{GFP} mice were analyzed during the time course of infection. Under the experimental conditions chosen, IAV infection caused maximum thymic involution at day 10 post infection accompanied by a significant increase in the frequency of thymic CD25^{+}Foxp3^{+} Tregs as well as their CD25^{-} Foxp3^{+} precursors. Interestingly, the absolute number of Tregs only slightly decreased during thymic atrophy, while the number of conventional CD25^{-}Foxp3^{-} thymocytes decreased significantly. Caspase 3/7 staining of newly-developing cells showed that these differences are likely not the result of preferential survival of Tregs, since both CD25^{+}Foxp3^{+} tTregs as well as CD25^{-}Foxp3^{-} thymocytes displayed an equal survival within atrophied thymi. Together, these results suggest that IAV infection does not lead to a depletion of Tregs, but rather accelerates their differentiation or promotes their retention within the thymus, while leading to an accelerated exit of conventional T cells. Currently, a mathematical model is being developed to test different hypotheses underlying the causes of thymic atrophy and its consequences on the peripheral T cell population.

P.A2.04.08

The RxR agonist Bexarotene promotes the induction of human iTregs, and reduces Th17 differentiation *in vitro*

***C. Gaunt**, A. Coles, J. Jones;*
University of Cambridge, Cambridge, United Kingdom.

Introduction: Retinoic acid (RA) promotes TGF-β-dependent differentiation of CD4+Foxp3+ iTregs from naïve CD4+ cells, and inhibits Th17 differentiation, by binding to the conditionally-permissive RA receptor (RAR)/retinoid X receptor (RXR) heterodimer. It is unknown if RXR agonists can modulate the iTreg/Th17 axis in humans, and whether this is dependent upon RAR-mediated signals. Here we investigate the effect of Bexarotene, a selective RxR agonist currently in trial as a multiple sclerosis re-myelinating therapy, on human iTreg/Th17 induction *in vitro*. **Materials and Methods:** Naïve CD4 cells from 15 healthy controls were cultured for 7 days in serum-free, and therefore RA-free media under: (i) iTreg (IL-2, TGF-β, anti-IFN-γ, anti-CD3/CD28) or (ii) Th17 (IL-2, TGF-β, IL-6, IL-1β, IL-23, anti-IFN-γ, anti-IL-4, anti-CD3/CD28) conditions ± Bexarotene (1 µg/ml), ATRA (40 nM), 9-Cis-RA (100 nM). At D7, the cells were harvested for immune-phenotyping and their ability to suppress the proliferative responses of naïve T cells to CD3/28 stimulation determined. Bisulphite sequencing was performed or sorted Foxp3+ cells to determine the methylation state of the TSDR (Treg specific demethylation region). **Results:** In the absence of RA, Bexarotene increased the differentiation of functionally suppressive iTregs, and decreased Th17 induction, from naïve cells. Similar effects were seen with RA. No additive effects were observed with RA and Bexarotene were combined. In keeping with the literature, iTregs induced in this study remained methylated at the TSDR. **Conclusions:** The RXR agonist Bexarotene alters the iTreg/Th17 axis in humans, in favour of iTreg induction. This supports a potential immune-regulatory role for Bexarotene.

P.A2.04.09

Study on the role of microRNA in the function of Treg-of-B cells

***Y. Huang**, C. Chien, B. Chiang;*
Graduate Institute of Immunology, Collage of Medicine, National Taiwan University, Taipei City, Taiwan.

Introduction: In our previous study, we found that splenic B cells could induce naïve CD4+CD25- T cells into CD4+CD25+Foxp3- regulatory T cells in cell-cell contact manner, termed Treg-of-B cells. Here, we aim to further study the microRNAs profile of Treg-of-B cells, and identify some significant microRNAs that might be engaged in the suppressive function, development or fitness on Treg-of-B cells.
Materials and Methods: Splenic B220+ and naïve CD4+CD25- T cells were purified from female BALB/c mice. We analyzed the surface molecules of Treg-of-B cells by flow cytometric analysis. T cell proliferative response with tritium-thymidine incorporation assay was used to assay Treg-of-B cells suppressive ability and ELISA to analyze cytokine secretion. TaqMan low-density array was used to study microRNAs expression profile, and TaqMan Q-PCR and northern blot were used for validation.
Results: The results suggested that Treg-of-B cells expressed regulatory-related surface markers and secreted regulatory cytokine. According to array data, it is indicated that certain microRNAs differentially expressed in Treg-of-B cells in the condition of fresh-isolation or stimulation. Then, we also studied its effect on the down-stream target genes. Most importantly, the impact of microRNAs on suppressive function of Treg-of-B cells will be further clarified in this research.
Conclusions: In our study, we assay the effect of microRNAs on target gene and suppressive function of Treg-of-B cells. With our efforts, we hope to have more information on the function and development of Treg-of-B cells and contribute to Treg-based therapies which might be applied for the treatment of immunological diseases.

P.A2.04.10

AR-CGD (p67 defect) with kabuki syndrome presenting Arnold Chiari malformation

***M. Y. Koker**[1], B. Saraymen[1], N. Köker[1], K. Boztug[2];*
[1]Erciyes medical school, Kayseri, Turkey, [2]Ludwig Boltzmann Institute, Medical University of Vienna, Vien, Austria.

we will present the first chronic granulomatous disease (CGD) case with kabuki syndrome. Our patient with autosomal recessive CGD (p67 defect) has also kabuki syndrome with Chiari malformation. Structural brain abnormality have only occasionally described in kabuki syndrome.Patient have an history of 2 cranial operation for Chiari malformation and clinical finding was resolved, but due to repeated infection history we do DHR assay for neutrophil function. DHR results showed that stimulation index was SI: 1 (no oxidase activity) which is specific for CGD. We did genetic analysis in this consanguineous family and we founded a nonsense homozygote mutation p.[Arg77X] in NCF2 gene. Additional genetic test like NGS have been done by Kaan Boztug lab and kabuki syndrome with heterozygote missense mutation (R132Q) in KMT2D gene was founded.Patient clinical condition is well and waiting for the bone marrow transplantation. Kabuki syndrome with Arnold chiari malformation is very rare clinical presentation. This male patient has also CGD, so it is the first case in the literature.

P.A2.04.11

Human CD4$^-$CD8$^-$ MAIT cells are a functionally and transcriptionally distinct subset that can be derived from the main CD8$^+$ MAIT cell pool

*J. Dias[1], C. Boulouis[1], J. Gorin[1], R. van den Biggelaar[1,2], K. G. Lal[1,3,4], A. Gibbs[5], L. Loh[6,7], M. Y. Gulam[8], S. Bari[9], W. Y. Hwang[9,10,11], D. F. Nixon[6,12], S. Nguyen[13], M. R. Betts[13], M. Buggert[1,13], M. A. Eller[3,4], K. Broliden[5], A. Tjernlund[5], J. K. Sandberg[1], **E. Leeansyah**[1,8];*

[1]Center for Infectious Medicine, Department of Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm, Sweden, [2]Department of Infectious Diseases and Immunology, Universiteit Utrecht, Utrecht, Netherlands, [3]U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [4]Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, United States, [5]Unit of Infectious Diseases, Department of Medicine Solna, Center for Molecular Medicine, Karolinska Institutet, Karolinska University Hospital Solna, Stockholm, Sweden, [6]Division of Experimental Medicine, Department of Medicine, University of California San Francisco, San Francisco, United States, [7]Department of Microbiology and Immunology, The University of Melbourne, Parkville, Australia, [8]Program in Emerging Infectious Diseases, Duke-National University of Singapore Medical School, Singapore, Singapore, [9]Department of Hematology, Singapore General Hospital, Singapore, Singapore, [10]National Cancer Centre Singapore, Singapore, Singapore, [11]Program in Cancer and Stem Cell Biology, Duke-National University of Singapore Medical School, Singapore, Singapore, [12]Department of Microbiology, Immunology, and Tropical Medicine, George Washington University, Washington, D. C., United States, [13]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, United States.

Mucosa-associated invariant T (MAIT) cells are a large subset of unconventional T cells that recognize microbial riboflavin metabolites presented by the MHC class I-like protein MR1. The majority of the human MAIT cell population either expresses the CD8α co-receptor and lacks CD4 (CD8$^+$), or is double-negative for CD4 and CD8 (DN). It is currently unclear if these subsets separated by CD8 expression are functionally distinct and if they represent distinct developmental lineages. Here, we show that the two MAIT cell subsets express distinct patterns of classical and innate-like T cell transcription factors and divergent transcriptional programs. CD8$^+$ MAIT cells have higher levels of receptors for IL-12 and IL-18, as well as co-stimulatory receptors. The differences in PLZF, RORγt, T-bet, and Eomes expression are more pronounced in mucosal tissue-derived CD8$^+$ MAIT cells. CD8$^+$ MAIT cells displayed superior functionality following stimulation with riboflavin-autotrophic and -auxotrophic strains of *Escherichia coli* or mitogens. Interestingly, DN MAIT cells from human fetal tissues and umbilical cord blood display a more mature phenotype and accumulate over gestational time with reciprocal contraction of the CD8$^+$ subset. Culture of CD8$^+$ MAIT cells in the presence of chronic T cell receptor stimulation leads to the accumulation of DN MAIT cells. Finally, DN MAIT cells are biased towards IL-17 production and have higher propensity for apoptosis. Overall, our study defines key differences in the transcriptional and functional profile between human CD8$^+$ and DN MAIT cells and their apparent derivative relationship.

P.A2.04.12

Functional diversity of cytotoxic- versus helper-type human CD8$^+$ memory T cells

***L. Loyal**[1,2], S. Warth[1], R. Stark[3], M. Frentsch[1], A. Thiel[1];*

[1]Regenerative Immunology and Aging, BCRT/Charité, Berlin, Germany, [2]International Max Planck Research School for Infectious Diseases and Immunology, Berlin, Germany, [3]Sanquin Blood Supply Foundation, Amsterdam, Netherlands.

CD4$^+$ memory T cells are organized into diverse T *helper* subsets such as Th1, Th2, Th17, Th17+1 and Th22 type cells characterized by distinct functions with highly specialized cytokine secretion and chemokine receptor expression patterns. Their differentiation is dependent on the cytokine milieu during activation inducing divergent differentiation programs based on the transcription factors TBX21, GATA3, RORC and AHR. In pathogenic settings, CD8$^+$ memory subsets secreting specialized cytokine combinations comparable to CD4$^+$ T *helper* subsets have been described too. We demonstrate that similar chemokine receptor patterns utilized for this T *helper* subset delineation are sufficient to systematically separate corresponding specialized CD8$^+$ memory T cell subsets Tc1, Tc2, Tc17, Tc17+1 and Tc22. These subsets exhibit differentiation-inducing transcription factor expression patterns comparable to the CD4$^+$ Th subsets as well as matching cytokine secretion profiles of IFNγ, IL-4, IL-13, IL-17, IL-22 and IL-10. While Tc1 and Tc17+1 display classical CD8$^+$ T cell related cytotoxic signature, Tc2, Tc17 and Tc22 cells possess a non-cytotoxic phenotype and express the CD4 *helper* molecule CD40L. Here we provide a fast and simple tool to identify the functionally divergent CD8$^+$ T cell subset directly *ex vivo*. We are applying this panel to systematic patient screenings (psoriasis, atopic dermatitis, MS, SLE) in order to identify relevant signatures of functionally distinct Tc subsets that contribute to the clinical profiles and provide useful information for potential treatment targets and agent effectivity.

P.A2.04.13

Distinct survival niches for memory T helper cells in spleen and bone marrow

***M. Mursell**, S. Hojyo, K. Tokoyoda;*

Deutsches Rheuma-Forschungszentrum, Berlin, Germany.

Introduction: CD4 T helper (Th) lymphocytes are an essential part of immunological memory. During the primary immune response, a subpopulation of antigen-experienced Th cells migrates into the bone marrow (BM) and resides there as the major population of memory cells in dedicated survival niches consisting of IL-7-expressing stromal cells. In addition to IL-7, IL-2 and IL-15 have been reported to contribute to the homeostasis of memory Th cells in the spleen or BM. However, molecular evidences have not been elucidated so far which factors are critical for the survival of memory Th cells in the spleen and BM.
Materials & Methods: To determine the roles of these factors, several (conditional) knock-out mice and antibodies were used in murine immune responses to protein antigens, mimicking systemic vaccines. Moreover, the co-localization of memory Th cells with cytokine-expressing cells was analyzed histologically in both spleen and BM using knock-in reporter mice.
Results: The interference of IL-7 signalling impaired the survival of splenic but not BM memory Th cells *in vivo*. IL-15 signalling had no impact on both memory cells. Interestingly, interference of the IL-2 signalling or depletion of regulatory T cells (Tregs) dramatically reduced the numbers of both memory cells. Histological analyses uncovered that Tregs indirectly contribute to the maintenance of memory Th cells.
Conclusion: Splenic memory Th cells require IL-7 and Tregs whereas BM cells depend on Tregs. The requirement of IL-7 distinguishes the survival niches for memory Th cells in the spleen and BM.

P.A2.04.14

Shaping effector and memory T cell differentiation through the immunosuppressive drug Leflunomide

***S. Scherer**[1], S. Oberle[2], D. Zehn[1];*

[1]Technical University of Munich, Freising, Germany, [2]Lausanne University Hospital, Lausanne, Switzerland.

Introduction: Leflunomide is an immunosuppressive drug frequently used to treat autoimmune diseases such as rheumatoid arthritis and multiple sclerosis. It is widely considered that it does so by inhibiting the proliferation of lymphocytes upon interfering with the de novo synthesis of pyrimidines. However, the exact mechanisms are not fully understood.
Materials and Methods: To address this issue we treated mice with Leflunomide during the course of an acute Listeria monocytogenes infection and monitored the virus specific CD8+ T cell response.
Results: We observed that Leflunomide treatment goes along with significant reductions in the numbers of pathogen-specific T cells. Interestingly, the residual T cells in treated mice were enriched for cells with a memory precursor phenotype while effector T cells were strongly reduced. Functional studies revealed that these precursor cells and the subsequently formed memory T cells are fully functional and we found that their global gene expression profiles were largely undistinguished from the corresponding cell populations formed in control mice. Moreover, we observed that T cells activated for 30 hours without Leflunomide proliferated normally when transferred into infected and Leflunomide treated mice.
Conclusions: Our results contrast the concept that Leflunomide simply causes proliferation arrest of T cells. Instead it suggests that Leflunomide blocks during the early T cell activation phase the acquisition of effector T cells programs while memory formation seems to be preserved. We see this treatment as a unique opportunity to ravel new mechanisms that determine the branching of CD8+ T cells into effector cells and memory precursors.

P.A2.04.15

Gestational age-dependent IgG glycosylation pattern in preterm infants

***N. Twisselmann**[1], Y. Bartsch[2], J. Pagel[1], M. Ehlers[2], C. Härtel[1];*

[1]Department of Child and Adolescent's Health, University of Lübeck, Lübeck, Germany, [2]Department of Nutritional Medicine, University of Lübeck, Lübeck, Germany.

Preterm infants acquire reduced amounts of immunoglobin G antibodies (IgG Abs) via trans-placental transport making them prone to development of infections in early life. The functional properties of IgG Abs are regulated by the Fc N-linked glycosylation patterns. Agalactosylated and asialylated Abs correlate with pro-inflammatory immune responses in e.g. rheumatoid arthritis, while galactosylated and sialylated IgG Abs mediate anti-inflammatory properties and increase during pregnancy. However, Fc glycosylation in preterm infants and their role in disease development are unknown.
We collected serum samples from n=83 infants with different gestational ages (23-34 weeks) and term infants as controls and additionally included n=18 mother-infants-pairs. Total IgG was purified and hydrolyzed with recombinantly expressed EndoS enzyme. The resulting N-glycan were purified and further investigated by high performance liquid chromatography.

The mother-infant-pairs show no difference in galactosylation and sialylation of the Fc N-linked glycan. The analysis of the gestational age-dependent glycosylation patterns revealed decreased galactosylated and sialylated Fc N-linked glycan with lower gestational age and increased agalactosylated and asialylated patterns. Within the highest risk group of 23 to 26 weeks of gestational age, there was no significant difference in Fc glycosylation in preterm infants developing sepsis or chronic lung disease.
The data suggest that Fc N-linked glycosylation is more prone to be pro-inflammatory with lower gestational age, but the transfer of Fc glycosylation is not selective. Further investigations with a bigger cohort are needed to determine the functional role in detail.

P.A2.04.16
Cellular survival niches for memory T helper cells in the spleen and bone marrow

***T. Wu**, S. Hojyo, K. Tokoyoda;*
DRFZ, Berlin, Germany.

Introduction: Immunological memory provides long-term protective immunity against pathogens that have been encountered before. Memory is maintained by memory cells generated in the primary challenge. We have so far shown that the majority of memory T helper (Th) cells are maintained as resting cells in IL-7$^+$collagen XI$^+$ stromal survival niches of the bone marrow (BM) and that B cells negatively regulate the generation of BM memory Th cells. However, the role of B cells in the maintenance of memory Th cells in the BM remains unknown. Here we show the role of CD20$^+$ cells in the maintenance of memory Th cells in the BM as well as spleen. **Method:** Serological ablation approach, e.g. anti-CD20, anti-IgD and anti-IgM treatments, was applied in mice generating memory Th cells. To identify the target cells, flow cytometry and confocal microscopy were used. **Results:** Deletion of CD20$^+$ cells expanded the numbers of memory Th cells in the spleen and BM, while deletion of IgD$^+$ and IgM$^+$ cells did not. Deletion of IgM$^+$ cells rather reduced the numbers of memory Th cells in the BM. **Conclusion:** CD20$^+$IgD$^-$IgM$^-$ cells negatively regulate the maintenance of memory Th cells in the spleen and BM, whereas IgM$^+$ cells support the maintenance of memory Th cells in the BM.

P.A2.04.17
Distinct Rap1 relays and donwstream pathways regulate T helper cell differentiation

***Y. F. Yazicioglu**[1,2,3], H. I. Aksoylar[3,4], R. Pal[3,4], N. Patsoukis[3,4], V. A. Boussiotis[3,4];*
[1]Department of Medicine, University of Heidelberg, Heidelberg, Germany, [2]Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey, [3]Division of Hematology-Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, United States, [4]Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, United States.

Rap1 is a small GTPase with a crucial role in canonical signaling events in hematopoietic cells. Integrin activation represents one of the best studied cellular processes in which Rap1 is implicated with its downstream effectors Rap1-interacting adaptor molecule (RIAM) and RapL. RIAM regulates integrin activation by altering the conformation of leukocyte function-associated antigen-1 (LFA-1) beta chain, whereas RapL interacts with LFA-1 alpha chain (LFA-1α). While the role of Rap1-mediated integrin activation in regulating migratory and homing properties of immune cells has been extensively studied, whether the Rap1 signaling axis regulates T helper lineage-decision as well as effector-memory T cell differentiation remains unclear. In this study, we employed mice expressing constitutively active Rap1-GTP in T cells with or without conditional knock-out of RIAM or LFA-1α. Using this approach, we aimed to investigate mechanistically the contribution of Rap1 signaling axis and its downstream effectors in T helper cell differentiation. Our results revealed that Rap1-GTP transgenic mice exhibited enhanced regulatory T (Treg), Th17 and T follicular helper (Tfh) cell differentiation in secondary lymphoid organs including Peyer's Patches under steady-state conditions. Endogenous effector-memory T cell pool was consistently enlarged in Rap1-GTP transgenic mice. We found that development of effector-memory and Foxp3+ regulatory T cells was primarily mediated by RIAM while Tfh differentiation was mediated through LFA-1α. Our results identify a novel function of Rap1 signaling in fine-tuning T helper differentiation and pave the way for further studies to dissect the role of specific Rap1 relays and downstream signaling pathways in T helper cell fate.

P.A2.04.18
Immunorehabilitology: Present and Perspectives. From Immunotherapy to Personalized Targeted Immunorehabilitation

***R. Sepiashvili**;*
Peopels Friendship University of Russia,RUDN University, Moscow, Russian Federation.

Development and introduction of modern clinical diagnostic tests (that allow to evaluate the functional system of immune homeostasis) into medical practice, a huge body of evidence on the leading role of the immune system in pathogenesis most acute and chronic diseases and even identification of specific nosological forms of immune-mediated diseases forced the scientists to search and develop new tools and techniques that have therapeutic effects on the impaired immune homeostasis and restore it to the normal state. The introduction of a novel concept - immunorehabilitation - was an impetus for the accumulation of new knowledge and a catalyst for research in clinical immunology. The first papers on this topic were published over 35 years ago by Revaz Sepiashvili who breathed life into the concept of immunorehabilitation. He was lucky to be at its origin. He became not only the founder of the brand new scientific field - immunorehabilitation, but also the founder of a new medical science - immunorehablitology.Immunorehabilitology is a research area concerned with the recovery of immune system functional activity to physiologically normal levels under the effect of complex systemic therapeutic and preventive measures (both pharmacological and non-medical ones) to provide the recovery from acute diseases or stable clinical immunological remission with minimal (or even without) recurrences in chronic conditions. In this paper, the author returns to the roots and recalls the way that medical science has gone before coming to understand immunorehablitology and tells about current successes and its development prospects.This publication was prepared with the support of the"RUDN University Program 5-100".

P.A2.04.19
Accumulation of functional multi-potent hematopoietic progenitors in peripheral lymphoid organs of mice over-expressing IL-7 and Flt3-ligand

*F. Klein[1], L. von Muenchow[1], G. Capoferri[1], S. Heiler[1], M. Mitrovic[1], C. Engdahl[1], L. Alberti-Servera[1], J. Andersson[1], R. Ceredig[2], A. Rolink[1], **P. Tsapogas**[1];*
[1]University of Basel, Basel, Switzerland, [2]National University of Ireland, Galway, Ireland.

Interleukin-7 (IL-7) and Flt3-ligand (FL) are two cytokines important for the generation of B cells, as manifested by the impaired B cell development in mice deficient for either cytokine or their respective receptors, and by the complete block in B cell differentiation in the absence of both cytokines. In order to further study their synergistic effect in lymphopoiesis *in vivo*, we generated mice constitutively over-expressing both IL-7 and FL. These double transgenic mice develop splenomegaly and lymphadenopathy characterized by tremendously enlarged lymph nodes even in young animals. We find a synergistic effect of the two cytokines in the expansion of bone marrow lymphoid and myeloid progenitors, including Lineage$^-$kit$^+$Sca1$^+$ (LSK), Common Lymphoid Progenitors (CLP) and pro/pre B cells, while Hematopoietic Stem Cells (HSC) are reduced by FL over-expression. Analysis of peripheral organs of these mice identified the presence of increased numbers of these progenitors in spleen and lymph nodes. When transplanted into irradiated wild-type mice, double transgenic lymph node cells show long-term multi-lineage reconstitution of hematopoietic lineages, further confirming the presence of functional hematopoietic progenitors therein. Our results provide further *in vivo* evidence for the concerted action of IL-7 and FL on lymphopoiesis and suggest that extra-medullary niches, including those in lymph nodes, can support the survival and maintenance of hematopoietic progenitors that under physiological conditions develop exclusively in the bone marrow.

P.A2.04.20
Relacja niskiego stopnia stanu zapalnego z ograniczeniem czynnościowym u osób starszych

***B. Morawin**, A. Tylutka, J. Chmielowiec, P. Rozpedowski, A. Zembron-Lacny;*
Faculty of Medicine and Health Sciences, Zielona Gora, Poland.

The age-related changes in skeletal muscle system are the result of chronic activation of macrophages, which leads to an increase in pro-inflammatory cytokines. The increase in circulating pro-inflammatory factors, such as TNFα, is far less than that seen in acute infection; thus the ageing effects on pro-inflammatory cytokine expression are considered to be a chronic low-grade state. The purpose of the study was to identify the inflammatory mediators responsible for deficit in functional fitness and to explain whether inflammation is related to changes in body composition and the decline of muscle strength in the elderly. Eighty women and men (71.2 ± 5.7 years) from the University of the Third Age (U3A) participated in the study. The pro-inflammatory cytokine TNFα (0.081 ± 0.036 pg/mL) as wells as soluble receptors sTNFR1 (154.75 ± 33.65 pg/mL) and sTNFR2 (331.66 ± 68.42 pg/mL) did not reached dangerously high levels. However, hsCRP concentration was significantly elevated (>3 mg/L) in the elderly with high fat mass (FM 33.3 ± 6.0 kg). The hsCRP level was correlated with FM (r=0.452, *P*<0.01). The result of 6-minute walking test was 442.7 ± 77.1 m, which shows good functional status in seniors from U3A. The elderly classified as obese and overweight were distinguished by low functional status and low isokinetic hand strength. The generation of inflammatory mediators in students of U3A was related to changes in body composition, isometric strength, and age-related changes in functional fitness. This study was financially supported by a project **2016/21/N/NZ7/03329** PRELUDIUM 11 from the National Science Centre, Poland

P.A2.04.21

Immunological changes in women and men over 60 years of age depending on coexisting disease entities

***A. Tylutka**, B. Morawin, J. Chmielowiec, A. Matejuk, A. Zembron-Lacny;*
Faculty of Medicine and Health Sciences, Zielona Gora, Poland.

p.p1 {margin: 0.0px 0.0px 0.0px 0.0px; font: 12.0px 'Times New Roman'; color: #212121; -webkit-text-stroke: #212121; background-color: #ffffff} span.s1 {font-kerning: none} span. s2 {font: 8.0px 'Times New Roman'; font-kerning: none}It is now known that the changes in the immune system in the elderly associated with the pool of T-lymphocytes consist mainly in the reduction of the number of naïve T cells and the increase in the number of memory lymphocytes and a reduction in the CD4/CD8 ratio <1. The aim of this study was to analyze cells of the immune system and the influence of physical activity on the improvement of immunological parameters. In research took part 99 students from The University of the Third Age and the control group (n=30) at the age of 21. U3A students were divided into two age groups, 60-70 and 70+. As a result of health questionnaires, the following disease entities were selected among the examined persons: arterial hypertension, diabetes, hyperthyroidism and hypothyroidism and rheumatoid arthritis. Using a flow cytometry a comparative analysis of cells with a phenotype $CD4^+CD45RA$ and $CD4^+CD45RO$, $CD8^+CD45RA$ and $CD8^+CD45RO$ as well as CD4/CD8 ratio was conducted.Statistically significant differences were observed between older and younger people in the $CD4^+CD45RO$ cell population ($p<0.0001$), $CD8^+CD45R0$ ($p<0.01$) and CD4/CD8 ($p<0.01$). Significance is also evident between active and inactive elderly in the population of $CD4^+CD45RA$ ($p<0.05$). The results of the study showed how physiological aging affects the population of immune cells, and additionally it has been proven that regular physical activity has a beneficial effect on the rejuvenation of the immune system.

P.A3.01 Immunomonitoring and biomarkers - Part 1

P.A3.01.01

Circulating hsa-miR-Chr8.96 biomarker discriminates acute autoimmune myocarditis and myocardial infarction Circulating hsa-miR-Chr8:96 biomarker discriminates acute autoimmune myocarditis and myocardial infarction

***R. Blanco-Dominguez**[1], R. Sánchez-Díaz[1], B. Linillos-Pradillo[1], H. de la Fuente[2], M. Relaño[1], L. Alonso-Herranz[1], K. Tsilingiri[1], I. García-Fernández[1,2], A. Martínez-León[3], M. Ricote[1], H. Bueno[4,1], L. Fernández-Friera[5,1], F. Alfonso[6], B. Ibáñez[1,7], F. Sánchez-Madrid[1,2], P. Martín[1];*
[1]Fundación Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain, [2]Servicio de Inmunología. Hospital Universitario La Princesa, Madrid, Spain, [3]Hospital Universitario Central de Asturias, Oviedo, Spain, [4]Hospital Doce de Octubre, Madrid, Spain, [5]HM Hospitales-Centro Integral de Enfermedades Cardiovasculares HM-CIEC, Madrid, Spain, [6]Servicio de Cardiología. Hospital Universitario La Princesa, Madrid, Spain, [7]IIS, Fundación Jiménez Díaz, Madrid, Spain.

The treatment and prognosis of myocarditis rely on an early diagnosis. However, acute myocarditis frequently mimics acute myocardial infarction (AMI) in its clinical presentation with different medical management and prognostic implications. There is a lack of a reliable tool for early differential diagnosis between these conditions. Using miRNA microarrays, we detect the expression of mmu-miR-721 mainly in Th17 cells, key players of myocardial damage in myocarditis. miRNA-721 is present in the plasma of mice with acute myocarditis, encapsidated into extracellular vesicles (EV) secreted by Th17 cells, but not in the plasma of mice with AMI. We identify hsa-miRNA-Chr8.96 as the miR-721 human homolog, that is selectively expressed into EV in the plasma of acute myocarditis patients upon clinical presentation. Analysis of the expression of hsa-miRNA-Chr8.96 in the EV-plasma compartment of 207 participants from five independent cohorts reveals a high potential diagnostic value of myocarditis patients compared to healthy controls (AUC: 0.9872) and AMI patients (AUC: 0.9652). Our data highlight hsa-miRNA-Chr8.96 as the first non-invasive biomarker for the diagnosis of acute myocarditis patients.

P.A3.01.02

Identification of high risk myelodysplastic syndrome via migration analyses of blood neutrophils - a diagnostic approach

*M. Schuster[1], M. Möller[2], **L. Bornemann**[1], C. Bessen[1], C. Sobczak[1], S. Schmitz[1], L. Witjes[3], A. Kündgen[2], N. Pundt[4], B. Pelzer[1], C. Ampe[3], M. Van Troys[3], R. Haas[2], U. Germing[2], L. Martens[3,5], K. Jöckel[4], M. Gunzer[1];*
[1]Institute for Experimental Immunology and Imaging, Essen, Germany, [2]Department of Hematology, Oncology and Clinical Immunology, Düsseldorf, Germany, [3]Department of Biochemistry, Ghent, Belgium, [4]Institute for Medical Informatics, Biometry and Epidemiology, Essen, Germany, [5]VIB-UGent Center for Medical Biotechnology, Ghent, Belgium.

Autonomous migration is essential for immune cells and has prognostic and diagnostic potential, yet clinical diagnostic lacks standardised migration assays. Here, we introduce a robust method to determine migration patterns of human neutrophils. We generated data from >130 healthy donors and compared them to patterns from patients suffering from myelodysplastic syndrome (MDS), whose majority show impaired neutrophil functions. In clinical routine, MDS diagnosis and prognosis is both time- and money-intensive and an easily quantifiable parameter would tremendously facilitate clinical work.
We established a standardised assay to analyse random 2-D migration of human neutrophils using time-lapse microscopy and automated cell tracking. Blood of healthy donors and MDS patients was provided by both, the Heinz-Nixdorf Recall MultiGeneration study (HNMGS) and the MDS registry, Düsseldorf, respectively.
Among the participants of the HNGMS, we found comparable baseline values with little variability concerning age group and sex. Individual values were highly reproducible and the response to known migration triggers was independent of the triggered receptor expression. Importantly, the migration pattern almost completely collapsed in patients suffering from severe types of MDS. In fact, induced neutrophil migration strongly correlated with the IPSS-R score, allowing a reliable identification of the high-risk cases.
In this study, we provide evidence that neutrophil migration is a quantifiable, reliable and minimally invasive readout for MDS prognosis. The long-term goal is to develop a robust platform for the routine testing of human leucocyte migration and identify migration-associated disease settings.

P.A3.01.03

Biomarkers of gut bariier damage in the early diagnostics of necrotizing enterocolitis

***S. Coufal**[1,2], A. Kokesova[3], H. Tlaskalova-Hogenova[1], J. Snajdauf[3], M. Rygl[3], M. Kverka[1];*
[1]Laboratory of Cellular and Molecular Immunology, Institute of Microbiology, CAS, v.v.i., Prague, Czech Republic, [2]Faculty of Science, Charles University, Prague, Czech Republic, [3]Department of Pediatric Surgery, 2nd Faculty of Medicine, Charles University, Motol University Hospital, Prague, Czech Republic.

Introduction: Necrotizing enterocolitis (NEC) is severe disease of gastrointestinal tract (GIT) affecting mainly preterm neonates and neonates after surgery for congenital malformation of GIT. Current NEC diagnostics does not allow to timely distinguishing NEC from other GIT disorders or sepsis. The aim was to test suitable biomarkers of gut barrier damage for early diagnostics of NEC. **Methods:** We included 42 infants with suspected NEC and 12 healthy infants as controls. The urine samples were collected in 6-hour intervals for 48 hours from the moment of NEC suspicion or after surgery for congenial developmental malformation of GIT. Serum samples were collected at the moment of NEC suspicion and one week later. Total and caspase cleaved cytokeratin-18 (CK-18), Trefoil factor 3 (TFF-3) and Intestinal-Fatty Acid Binding Protein (I-FABP) were measured using ELISA. **Results:** Individuals suffering from NEC had significantly higher I-FABP levels than individuals suffering from sepsis or healthy individuals.
There was significant decrease of I-FABP during NEC therapy. The levels of TFF-3 were significantly elevated both in infants suffering from NEC or sepsis in comparison with healthy individuals. The levels of CK-18 did not differ among groups. There were no significant differences in biomarkers levels between infants with spontaneous or surgery related NEC. By addition of the I-FABP examination to the standard NEC diagnostics we revealed 9 radiologically and ultrasonographically negative patients, who developed NEC later.
Conclusion: I-FABP is suitable biomarker for the early diagnostics of both spontaneous and surgery related NEC. Supported by IGA 13483, GAUK 326815, AZV 15-28064A.

P.A3.01.04

A comparative study between anti-endomysial antibodies and anti-transglutaminase antibodies in the management of celiac pediatric patients.

***C. García-Miralles**, J. Delgado, M. Amengual;*
Parc Taulí Hospital Universitari. Institut d'Investigació i Innovació Parc Tauli I3PT, Sabadell, Spain.

Introduction: Anti-transglutaminase antibodies (tTG) and anti-endomysial antibodies (EMA) are used as immunological markers for the diagnosis of celiac disease (CD). The objective of this study is to verify the concordance between anti-tTG and EMA in the diagnosis and follow-up of CD.
Methods: Retrospective study of the 2015-2017 period with the inclusion of 2267 pediatric patients who underwent the determination of EMA by immunofluorescence (INOVA®) and the detection of anti-tTG by ELISA (Immunocap®). To verify concordance, anti-tTG cut-off point was established by using a ROC curve, in which 300 patients were included: 150 patients with anti-tTG values >3 U/mL and negative EMA, and 150 patients with anti-tTG values >3 U/mL and positive EMA. ATA negative values (<3 U/mL) were excluded.
Results: The anti-tTG cut-off point was stablished at 7.1 U/mL, with a sensitivity of 87% and a specificity of 92%. During this period, 3055 analytical test of 2267 patients were analyzed, 2715 negative and 225 positives by both methods (96.2% concordance). Regarding the discrepancies, 100 serum samples were positive for EMA and negative for anti-tTG. Four of those patients were diagnosed of CD. On the other hand, 15 serum samples were positive for anti-tTG and negative for EMA. Two of those patients were diagnosed of CD.
Conclusion: The screening of both EMA and anti-tTG allowed the diagnosis of CD to 6 patients who had serological discrepancies, to whom the intestinal biopsy was avoided. Both methods behave similarly in the diagnosis and follow-up of CD.

P.A3.01.05

Protein biomarkers predictive of multivalent response to a novel dengue vaccine

*V. Hindie[1], P. Croteau[1], S. Aziri[1], S. Lo[1], J. Sui[1], L. Cortes[1], E. Kallas[2], R. P. Sekaly[3], **E. Paramithiotis**[1];*
[1]CAPRION BIOSCIENCES INC., MONTREAL, Canada, [2]University of Sao Paulo, Sao Paulo, Brazil, [3]Case Western Reserve University, Cleveland, United States.

Dengue fever is the most prevalent mosquito-borne disease of humans, and is caused by the dengue virus (DENV) which is comprised of four serotype groups that circulate globally. Protective antibodies against one DENV serotype offer limited protection against the others. Therefore, a vaccine that protects against all four serotypes has been highly desirable. We analyzed plasma from a Phase II trial of TV003, a live-attenuated vaccine composed of all four DENV serotypes, to identify predictive biomarkers of vaccine efficacy. Pre-dose, day 6 and day 15 post vaccination timepoints were analyzed from seronegative (n=17) or seropositive subjects (n=18). The samples were depleted of abundant proteins and processed to tryptic peptides for multiple reaction monitoring mass spectrometry (MRM-MS) using a previously defined assay composed of 298 host plasma proteins associated with the immune response. Overall protein detection rate was 96% with a study wide 9% median CV of the process controls used and 71% of the targeted proteins were detected in >50% of the samples. Supervised and non-supervised study sample grouping and network analysis of the differentially expressed proteins was done. Oligovalent responders had consistently elevated expression of pro-inflammatory proteins at baseline and later timepoints compared to multivalent responders, and network analyses indicated that the IL6 and acute phase response pathways were induced. In contrast, multivalent responders at baseline had comparatively elevated cellular activation, proliferation, and migration as well as neutrophil function. These results suggest that baseline inflammation can reduce multivalent DENV serotype response. Funded by NIH/NIAID and the Butantan Institute.

P.A3.01.06

T cell receptor signatures in cytomegalovirus carriers

***A. Huth**[1], X. Liang[1,2], S. Krebs[3], H. Blum[3], A. Moosmann[1];*
[1]DZIF Research Group "Host Control of Viral Latency and Reactivation" (HOCOVLAR), Helmholtz Center Munich, Munich, Germany, [2]HRYZ Biotech Co., Shenzhen, China, [3]Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, LMU Munich, Munich, Germany.

The herpesvirus human cytomegalovirus (CMV) causes a persistent infection in its host. Primary infection and reactivation can cause severe diseases in immunodeficient carriers, who cannot keep the virus in check. T cells are crucial for control of the virus and the presence of specific T cells is associated with protection against CMV disease.
We characterized the highly diverse T cell receptor (TCR) repertoire that humans mobilize to fight CMV. Virus-specific T cells were enriched by stimulation of blood cells from healthy donors with defined CMV epitope peptides. We performed high-throughput TCRβ sequencing, and subsequently compared TCR read frequencies in the stimulated sample compared to the unstimulated and a control peptide stimulated sample. In this way, we identified hundreds of CMV-specific TCRs against different CMV epitopes.
Enrichment of virus-specific T cells was exclusive to CMV-positive donors. The T cell response was often dominated by one or a few TCR clonotypes. Several TCR sequences with identical specificity were highly similar on the amino acid level and expressed by multiple donors. The cumulative *ex vivo* frequency of these TCR families was considerably higher in CMV-positive than CMV-negative individuals.
Virus-specific TCR families shared between virus carriers will be valuable in disease monitoring, for instance as an indicator of the presence of CMV-specific T cells. In addition, such TCRs are predestined to be used for adoptive T cell transfer, since they are tolerant to a wide range of HLA-self peptide complexes and are therefore less likely to cause autoimmunity in the recipient.

P.A3.01.07

Intake of soy isoflavones and vitamin D decreased inflammation, fecal serine protease activity, and had no effect on antioxidants in irritable bowel syndrome

***M. Jalili**[1], H. Vahedi[2], H. Poustchi[2], A. Hekmatdoost[3];*
[1]CMBG, BI, NTNU, Trondheim, Norway, [2]TUMS, Tehran, Iran, Islamic Republic of, [3]SBMU, Tehran, Iran, Islamic Republic of.

Introduction Irritable bowel syndrome (IBS) is a common gastrointestinal disorder in women. Isoflavones and vitamin D can regulate the estrogen receptors in colon, so our objective was to study the effect of isoflavones, vitamin D, and co-administration of them on inflammation markers, intestinal permeability and antioxidant status in women with IBS.
Materials and Methods Eligible IBS patients were allocated randomly to four groups: isoflavones (40 mg) +placebo of vitamin D (I+P), vitamin D (50000 IU) +placebo of isoflavones (D+P), isoflavones and vitamin D (I+D), and placebos of isoflavones and vitamin D (P+P). In a double-blind randomized clinical trial, 100 participants received treatments for 6 weeks. Five ml blood and fecal samples was taken at week0 and 6, plasma separated and tumor necrosis factor-α (TNFα), nuclear factor κβ (NFκβ), total antioxidant capacity (TAC) were measured. Serine protease (SrPr) activity was determined in feces. One-way ANOVA used to compare groups and $P<0.05$ considered as significance level.
Results Plasma TNFα, NFκβ and fecal SrPr was reduced in all treatment groups compared to placebo group, however the reduction level was statistically significant in all three treatment groups for NFκβ and SrPr ($p<0.001$), in I+P and I+D groups for TNFα ($P=0.003$ and 0.002 respectively). There were no significant changes in TAC levels.
Conclusion administration of soy isoflavones alone or with vitamin D reduced plasma inflammatory markers and improved intestinal permeability index by lowering fecal SrPr activity.

P.A3.01.08

Alterations in natural Dendritic Cell (DC) subsets in advanced cancer patients.

***A. Kaur**, J. Adhikaree, H. Franks, P. Patel, A. M. Jackson;*
Division of Cancer and Stem Cells, School of Medicine, University of Nottingham, Nottingham, United Kingdom.

The activity of natural DC is key for invoking immunity and for the success of immune checkpoint blockade for advanced cancer patients. Nivolumab and Ipilimumab block the inhibitory receptors namely PD-1 and CTLA-4 receptors on T cells allowing them to restore their anti-cancer functions. An understanding of the condition of natural DC subsets in these patients will assist in early stratification of responders, and may yield approaches to enhance the function of DC in other patients. We therefore measured the abundance and phenotype of 4 circulating DC subsets in cancer patients using a multi-parametric flow cytometry assay and compared it with data obtained from healthy controls. This assay has been performed for 16 patients with glioblastoma, 5 patients with advanced melanoma (currently on checkpoint inhibitor therapy) and 19 matched healthy donors. The average number of DC subsets in patient blood compared to healthy donor samples were 4476 vs 5056 of CD1c+ DC/mL, 193 vs 252 of CD141+ DC/mL, 1265 vs 2957 of plasmacytoid DC/mL (p-value=0.002) and 5760 vs 4265 of SLAN DC/mL respectively. A significant reduction in particular was observed in blood plasmacytoid DC subset in cancer patients as compared to healthy donors ($P<0.05$). As number of DC are important to efficiently activate the T cells after checkpoint inhibitor treatment, we believe that the number and phenotype of DC subsets may differ among responding and non-responding patients and this could further help identify the patients who would benefit the most with the therapy.

P.A3.01.09

Prognostic value of elevated T helper 17 and T helper regulatory cells related inflammatory serum markers in pathogenesis of septic polytrauma patients.

***S. Khurana**, P. Mathur, M. Kumari, N. Bhardwaj, S. Sagar, S. Kumar, A. Gupta, R. Aggarwal, K. Soni, R. Malhotra;*
All India Institute of Medical Sciences, New Delhi, New Delhi, India.

Introduction: Sepsis is a fatal immunological disorder and its pathophysiology remains poorly understood. It leads to high mortality rates, increased hospital stays and associated costs, especially in trauma patients. The present study was conducted to ascertain the role of T-helper lymphocyte-related inflammatory serum cytokines in trauma patients with blood culture positive with gram negative bacteria.
Materials & Methods: This study was conducted in a cohort of 40 polytrauma patients, bead-based cytometric analysis was used to quantify extracellular levels of sixteen serum cytokines using the multi-analyst kits using flowcytometry, in the serum on the day of obtaining positive blood culture and on day 4, post antimicrobial treatment. The cytokine profiles were compared with those in equal number of healthy controls and then correlated to clinical outcomes.
Results: A total of 40 patients and 40 healthy controls were included in the study. Of these, 24 patients (60%) were discharged while 16 (40%) had a fatal outcome. Statistically significantly elevated levels of serum IL-6, IFN-γ, TNF-α, IL-17A, IL-17F, and IL-4 were observed in septic patients, while lowered IL-13 levels correlated significantly with favourable outcome in these patients.
Conclusion: An elevated T helper17 and T helper regulatory cytokine response was observed. The unique cytokine profile of septic polytrauma patients is still poorly understood. More studies with larger sample size and stringent exclusion criteria are needed to be able to answer the question of identifying a reliable cytokine profile for prognostication of high risk septic trauma patients.

P.A3.01.10

A novel whole blood assay for detecting and profiling vaccine-induced *Bordetella pertussis* specific T-cells

N. Lambert[1], *V. Corbière*[2], *J. van Gaans*[1], *M. Duijst*[1], *E. Simonetti*[3], *E. van Schuppen*[3], *D. Diavatopoulos*[3], *A. Misiak*[4], *K. Mills*[4], *P. Versteegen*[1], *G. Berbers*[1], *C. van Els*[1], *F. Mascart*[2];
[1]*National Institute of Public Health and the Environment, Bilthoven, Netherlands,* [2]*Université Libre de Bruxelles, Brussels, Belgium,* [3]*Radboud University Medical Center, Nijmegen, Netherlands,* [4]*Trinity College Dublin, Dublin, Ireland.*

Introduction: Pertussis, a severe respiratory infectious disease caused by the bacterium *Bordetella pertussis* (*Bp*), remains endemic despite vaccination. No correlates of protection have been identified yet. *Bp*-specific CD4+CD3+IFNg+ T-cells are thought to play an important role in durable protection against infection and transmission. Novel sensitive and robust assays are needed to study the magnitude and differentiation of (vaccine-induced) antigen-specific T-cells in order to advance the development of improved pertussis vaccines.
Materials and methods: In a Dutch clinical study, 11-15 year-old healthy volunteers were boosted with an acellular pertussis vaccine. Whole blood samples, taken at day 0 (pre-booster), 14 and 28 (post-booster) were stimulated with *Bp* antigens overnight and production of cytokines was measured intracellularly and in supernatants by flow cytometry.
Results: Following vaccination, *Bp*-specific Th1 and/or Th2-cytokine producing T-cells increased to 0.02-0.5% of the CD4+CD3+ T-cell population. Supernatant analyses are ongoing. Standardized analysis of flow cytometric data is being explored. Currently, inclusions are being finalized and overall results will be presented.
Conclusions: We have shown that our novel whole blood assay is capable of detecting low frequency T-cell responses against *Bp*. Further analysis is ongoing to assess the Th1/Th2/Th17-profiling capacity of the assay and whether different T-cell profiles can be detected in booster responses of differently primed teenagers.
The PERISCOPE project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 115910. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programma and EFPIA and BMGF.

P.A3.01.11

IFNγ-preconditioning treatment increases immune system-related proteins in extracellular vesicles derived from human endometrial mesenchymal stromal cells

F. Marinaro[1], *F. Sánchez-Margallo*[1], *V. Álvarez*[1], *R. Blázquez*[1], ***E. López***[1], *B. Macías-García*[1], *M. Gómez-Serrano*[2], *I. Jorge Cerrudo*[2], *J. Vázquez*[2], *J. García-Casado*[1];
[1]*Centro de Cirugía de Mínima Invasión, Cáceres, Spain,* [2]*Centro Nacional Investigaciones Cardiovasculares CNIC, Madrid, Spain.*

Introduction: Endometrial Mesenchymal Stromal Cells (endMSCs) are multipotent cells with clear immunomodulatory effects. These cells release extracellular vesicles (EV-endMSCs), although their role in immune and inflammatory response is still under investigation. Here we show the proteomic profile of EV-endMSCs together with the effect of IFNγ-preconditioning.
Materials and Methods: EndMSCs were isolated from human menstrual blood, expanded *in vitro* and characterized by flow cytometry (n=3). EndMSCs were treated with IFNγ (3ng/ml) for 6 days, IFNγ was removed and supernatants were collected after 4 further days. EV-endMSCs were isolated from supernatants and protein extracts underwent high-throughput multiplexed quantitative proteomics based on iTRAQ labeling and mass spectrometry analyses. Enrichment analyses, using Gene Ontology (GO) and Reactome databases, were performed to evaluate the functional classification of the proteins identified.
Results: EndMSCs expressed CD56, CD44, CD29, CD90, CD105 and CD73 stemness markers, while EV-endMSCs expressed the exosome-related proteins CD9 and CD63. In the proteomic profiling, 1802 proteins were identified and 856 proteins belonged to GO term "extracellular vesicle" ($p<0.01$). Quantitative analyses showed 55 upregulated proteins after IFNγ treatment ($p<0.05$). Enrichment analyses revealed that 26 proteins were associated to the Innate Immune Response (i.e. M-CSF, C1Inh, STAT1) and 11 proteins to the Adaptive Immune Response (i.e. Cathepsin S, CD166 antigen, Beta-2-microglobulin). Moreover, 23 out of the 55 proteins were also related to the Reactome category "Immune system".
Conclusions: EV-endMSCs proteomic profiling reveals that IFNγ treatment upregulates immune system-related proteins, revealing their theurapeutic potential for the treatment of immune-mediated diseases.

P.A3.01.12

Fecal calprotectin interlaboratory comparisons

M. Martin[1], *M. López-Hoyos*[2];
[1]*Centro de Hemoterapia y Hemodonación de Castilla y León, Valladolid, Spain,* [2]*Hospital Universitario Marqués de Valdecilla, Santander, Spain.*

Calprotectin is a dimer of calcium binding proteins mainly present in neutrophil's cytosol. Elevated fecal calprotectin happens as a consequence of the migration of neutrophils to the intestinal mucosa due to any intestinal inflammation. Taken together with clinical signs and symptoms, this non-invasive test helps avoiding colonoscopies. Two rounds of an interlaboratory comparison were run along 2017, shipping stool aliquots from 4 different patients to 21 clinical labs. Samples were given after informed consent in HU Marques de Valdecilla. Each participant lab analyzed samples by their routine method. Both qualitative and quantitative results were reported. Robust statistics for interlaboratory comparison were performed following guidelines in ISO 13528. Three out of the 4 samples were assigned positive by consensus of at least 75% of participants, whereas the 4th sample remained inconclusive so far only a 47% of labs reported it positive. It was reported to contain 280 µg/g calprotectin. Samples yielding positive results were reported to 344-3819 µg/g calprotectin. Some patients have been reported to have slightly raised levels of fecal calprotectin, who would only need monitoring. Those of them suffering from a low-grade inflammatory bowel disease (IBD) will usually evolve to higher calprotectin levels. It will be therefore useful for labs and interlaboratory comparisons can help narrowing their grey zones.

P.A3.01.13

Flow cytometry: identifying biomarkers for the diagnosis of organ-specific autoimmune diseases and response to treatment

A. Teniente-Serra[1], *M. Fernández*[2], *B. Soldevila*[1], *E. Pizarro*[3], *C. Ramo-Tello*[1], *R. Pujol-Borrell*[4], ***E. Martínez-Cáceres***[1];
[1]*Germans Trias i Pujol University Hospital, Badalona, Spain,* [2]*Germans Trias i Pujol Research Institute (IGTP), Campus Can Ruti, Badalona, Spain,* [3]*Hospital de Mataró, Mataró, Spain,* [4]*Hospital Universitari Vall d'Hebron, Barcelona, Barcelona, Spain.*

In the last years, the significant development in the field of biomedical research has lead to the need to define new biomarkers for diagnosis, prognosis or monitoring of diseases. Multiparametric flow cytometry has been positioned as one of the most useful technologies for monitoring immune-mediated diseases.
For this purpose we designed an exhaustive flow cytometry panel which allows to analyse minor lymphocyte subpopulations in peripheral blood, and validated it in several autoimmune diseases:
1) We found changes in peripheral blood lymphocyte compartments of type 1 diabetes patients at onset of the disease.
2) In Graves' disease patients, a different pattern of lymphocyte subpopulations was identified in patients clinically stable who maintain the presence of anti-TSH autoantibodies, compared to those without autoantibodies.
3) In Multiple sclerosis, we found changes in lymphocyte subpopulations identified in untreated relapsing-remitting patients and progressive forms compared with healthy donors. The influence of immunomodulatory therapies on lymphocyte subpopulations was also analysed in a cross-sectional study.
Analysing the influence of therapies on lymphocyte subpopulations, we identified in a prospective study that Multiple sclerosis patients treated with fingolimod had different patterns of subpopulations, able to discriminate responders versus non-responders to the therapy , in a 12 month follow-up.
In conclusion, characterization of minor lymphocyte subpopulations in peripheral blood, by multiparametric flow cytometry, is a useful tool to identify potential biomarkers for the diagnosis and response to treatment of organ-specific autoimmune diseases, and by extension to other immune-mediated diseases.

P.A3.01.14

Peptidylarginine deiminase 4 gene polymorphisms associate with systemic lupus erythematosus and lupus nephritis

L. Massarenti[1,2], *D. Damgaard*[1,3], *C. Enevold*[1], *N. Ødum*[2], *C. H. Nielsen*[1,3], *S. Jacobsen*[4];
[1]*Institute for Inflammation Research, Center for Rheumatology and Spine Disease, section 7521, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark,* [2]*Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark,* [3]*Section for Periodontology, Department of Odontology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark,* [4]*Copenhagen Lupus and Vasculitis Clinic, Center for Rheumatology and Spine Diseases, section 4242, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark.*

Objective Deposition of immune complexes containing DNA and nuclear proteins, released during neutrophil extracellular trap (NET) formation, plays a key role in the pathogenesis of systemic lupus erythematosus (SLE) and lupus nephritis (LN). Histone citrullination, catalyzed by peptidylarginine deiminase 4 (PAD4), allows chromatin decondensation, and is thereby essential for NETosis. We sought to determine if selected single nucleotide polymorphisms (SNPs) in the encoding gene, *PADI4*, previously known to influence expression and functionality of the enzyme, affect risk of developing SLE and LN. **Methods**: 234 patients and 484 controls were genotyped for nine *PADI4*-SNPs using an in-house assay. Analyses were adjusted for age and gender. **Results**: Homozygosity and heterozygosity for rs1635564(T) were associated with increased SLE occurrence (P=0.02, OR 1.52, 95% CI 1.06-2.19 and P=0.03, OR 2.06, 95% CI 1.08-3.93, respectively), and homozygosity for rs1635564(T) was associated with increased LN occurrence (P=0.03, OR 3.35, 95% CI: 1.2-10.97). Notably, a gene dose effect for rs1635564(T) was observed for SLE and LN (P=0.005, OR 1.47, 95% CI 1.12-1-93 and P=0.01, OR 1.74, 95% CI 1.13-2.68, respectively). Additionally, minor allele carriage of five other SNPs (rs11203366, rs11203367, rs874881, rs2240340 and rs11203368) in high linkage disequilibrium, was associated with increased occurrence of LN and hypertension. **Conclusion**: We provide the first indications that the rs1635564 polymorphism of *PADI4* could be a risk factor for SLE, in general, and LN in particular. Additional *PADI4* polymorphisms may confer increased risk of LN. Overall, these findings support the notion of a role for citrullination in SLE and LN pathogenesis.

P.A3.01.15

Splenic CD4+CD39+ T lymphocyte frequencies are differentially altered in patients with leukemia and autoimmune thrompocytopenia

N. Nanava[1], *S. Metreveli*[1], *N. Kikodze*[2], *G. Giorgobiani*[3], *T. Chikovani*[1], *N. Janikashvili*[1];
[1]*Department of Immunology, Tbilisi State Medical University, Tbilisi, Georgia,* [2]*Institute of Medical Biotechnology, Tbilisi State Medical University, Tbilisi, Georgia,* [3]*Department of Surgery, Tbilisi State Medical University, Tbilisi, Georgia.*

Introduction: Accumulating evidence suggests that the expression of ectoenzime CD39 in CD4+ T lymphocytes indicates on their suppressive acitvities. In certain immune pathologies - blood cancer or autoimmune hematological disorders, there is a direct relationship between circulating and splenic T cell biomarkers reflecting the response to therapy. Our retrospective study aims to explore the differential expressions of CD39 and FoxP3 in blood and splenic CD4+ T cells of patients with chronic myelogenous leukmeia (CML) and immune thrombocytopenia (ITP). Individuals undergoing splenectomy due to other reason than cancer or autoimmune disease are used as controls.
Methods: Mononuclear cells from peripheral blood and dissociated spleen tissue are purified and the expressions of CD39 and FoxP3 are quantified within the CD4+ compartment of T lymphocytes. Data are acquired on a FacsCalibur flow cytometer and analyzed using FlowJo® v10 software.
Results: Our data suggest that the frequency of total circulating as well as splenic CD4+ T lymphocytes are comparable between CML and ITP patients and controls. However, CD39 expression is elevated in these T cells in patients with myelogenous leukemia and diminished in patients with ITP. Importantly, the differential expression of CD39 resembles FoxP3 levels in both groups of patients and is more evident in spleen compared to blood.
Conclusion: Expression of CD39 in splenic CD4+ T cells can be considered as a good biomarker and, respectively, a prospective therapeutic target in patients with CML and ITP undergoing therapeutic splenectomy.
This research was funded by Shota Rustaveli National Science Foundation (Grant №PhD_F_17_20).

P.A3.01.16

Granzyme B induced by RV0140 antigen better classified latently infected from active tuberculosis patients

R. Ouni[1,2], *A. Braïek*[1,3], *V. Dirix*[4], *H. Gharsalli*[5], *A. Jarraya*[6], *N. Sendi*[1], *A. Baccouche*[1], *A. Akremi*[6], *L. Gharbi-Douik*[5], *R. Barbouche*[1], *C. Benabdessalem*[1];
[1]*Laboratory of Transmission control and immunobiology of infections, Institut Pasteur de Tunis, Tunis, Tunisia,* [2]*faculty of sciences of Bizerte, University of Carthage, Bizerte, Tunisia,* [3]*University of Tunis El Manar, Tunis, Tunisia,* [4]*Laboratory of vaccinology and mucosal immunity, Université Libre de Bruxelles, Brussels, Belgium,* [5]*Hospital of Abderrahman Mami, Ariana, Tunisia,* [6]*Dispensaire anti-TB Ariana, direction régionale de la santé de l'Ariana, Ariana, Tunisia.*

Nearly two billion people are latently infected with *M.tb* (LTBI). Detection of LTBI with high risk to develop active tuberculosis (aTB) is considered the corner stone to control the disease. The current challenge is to identify markers that better classified LTBI from aTB. It has been shown that Rv0140, a reactivation-associated antigen of Mtb, induced significantly higher IFNg in LTBI individuals as compared to aTB. Herein, we described that Rv0140 induces high GranzymeB level mainly by PBMC derived from LTBI (n=33) as compared to aTB (n=18). ROC curves were used to evaluate the capacity of Rv0140 specifically induced IFNg and GranzymeB levels to classify LTBI from aTB. Our results show that, in response to Rv0140, GranzymeB allowed better discrimination of LTBI from aTB with areas under the curve (AUC) of 0.88 (95% CI 0.77-0.98) as compared to IFNg, AUC of 0.85 (95% CI 0.74-0.96).When combining GranzymeB and IFNg the AUC reaches 0.96. In the current study, we show that GranzymeB could be considered as another biomarker of TB that could be used as an alternative or in adjunction of IFNg to better classified LTBI from aTB.

P.A3.01.17

Long-term immunologic effects of human endotoxemia: similarities and differences with sepsis

Y. Rodriguez Rosales[1], *M. Kox*[2], *E. van Rijssen*[1], *M. van Welie*[1], *P. Pikkers*[2], *I. Joosten*[1], *H. Koenen*[1];
[1]*Laboratory of medical immunology, Radboudumc, Nijmegen, Netherlands,* [2]*Intensive care unit, Radboudumc, Nijmegen, Netherlands.*

Introduction: Sepsis is the cause of more than 5.3 million deaths per year, and novel immunotherapeutic strategies are highly warranted. Human models that mirror sepsis immunology are instrumental to this aim. The response to endotoxin administration in humans during the first 24 hours, captures many hallmarks of the inflammatory response observed in early sepsis. However, the long-term immunologic effects of human experimental endotoxemia have been sparsely studied and could be determinant for the use of this human model in sepsis therapy research. Aim and methods: We studied the immune cell composition of healthy subjects challenged with a bolus of endotoxin (1 ng/kg) 4 hours, 2 days and 20 days' post-administration by flow cytometry to study the effects on the innate and adaptive immune system, and compared it with the immune cell composition in patients during the first 9 days after onset of septic shock. Results: As a result of experimental endotoxemia, an increase in absolute numbers of intermediate monocyte was observed, which also showed lower HLA-DR expression 20 days' post-endotoxin. These changes differed with those observed in septic shock patients. Another long-term effect of experimental endotoxemia was elevated numbers of effector CD8+ cells and an increased percentage of proliferating and cytokine expressing CD8+ cells, these phenomena were also present in sepsis patients. Conclusion: We propose that experimental endotoxemia can be used to study several aspects of adaptive immunity in sepsis, specifically the behavior of CD8+ T-cells, which may eventually aid the development of new therapies for sepsis patients.

P.A3.01.18

Clinical, demographic and laboratory data associated with the risk of progressive multifocal leukoencephalopaty risk in multiple sclerosis patients treated with natalizumab

I. Toboso, A. Tejeda, S. Saiz, R. Alvarez-Lafuente2 , R. Arroyo3 , H. Hegen4 , ,G. Izquierdo5 , D. Paramo5 , P. Oliva6 , B. Casan, J. Álvarez, ***L. Villar****;*
Ramón y Cajal Hospital, Madrid, Spain.

Introduction: Natalizumab is and effective treatment for relapsing-remitting multiple sclerosis (MS). However, the risk of progressive multifocal leukoencephalopathy (PML), a serious opportunistic infection by JC virus limits its use. Anti JC antibodies, prior immunosupresion and time on natalizumab are risk factors for PML onset. We aimed to identify new biomarkers predicting PML onset in MS patients treated with natalizumab. Patients: We studied 1240 MS patients treated with natalizumab. Thirty-five developed PML. Clinical and demographic variables were studied. Moreover, lipid-specific oligoclonal IgM bands (LS-OCMB) were explored in a subgroup of 277 patients by isoelectric focusing and western blot. Data were analyzed with the Stata statistical package. We used MannWhitney U tests, ROC curves, and uni and multi variate logistic regression analyses to study PML risk. Results: We confirmed that anti-JC antibodies predicted PML risk. However, no effect of prior immunosuppression or time on natalizumab were observed in this cohort. New variables were identified in our study. The strongest association with PML risk were a relapse rate below 0.5 prior to natalizumab ($p<0.0001$) and an age over 45 years (p=0,048). Those were the only variables that remained significant in the multivariate analysis of the total cohort (AUC=0.85). We also confirmed the association of LS-OCMB and lower PML risk ($p<0.0001$). In junction with relapse rate below 0.5 and age over 45 constituted an accurate model for PML risk stratification (AUC=0.92). Conclusion: We describe here an statistical model that can contribute to establish PML risk in individual MS patients treated with natalizumab.

P.A3.01.19

Mass cytometry and immune cell-specific gene expression analysis of matched psoriatic arthritis blood and synovial fluid

N. Yager[1], *S. Cole*[2], *A. Lledo Lara*[1], *A. Maroof*[2], *C. Simpson*[2], *P. Bowness*[1], *H. Al-Mossawi*[1];
[1]*University of Oxford, Oxford, United Kingdom,* [2]*UCB Pharma, Slough, United Kingdom.*

Introduction: Mass Cytometry (CyTOF) has revolutionised the way cell samples can be immunophenotyped, allowing simultaneous quantification of >30 parameters with minimal spillover. This study aims to use CyTOF to generate a high-dimensional data-set of immune populations in matched psoriatic arthritis (PsA) blood and synovial fluid directly *ex vivo*, followed by transcriptomic analysis of key expanded cell populations.
Materials and Methods: Paired blood and synovial fluid were taken from 10 PsA patients and fixed with formaldehyde within 30 min of venipuncture/aspiration. The cells were stained with a 34-channel CyTOF panel of surface markers. Analysis was performed using conventional biaxial manual gating and unbiased visualisation methods including PhenoGraph and SPADE. RNA was isolated from the core expanded populations of freshly cell sorted PBMC and synovial fluid samples (n = 3), and a 384-gene array was performed.
Results: Using only one staining panel and applying multiple clustering algorithms, we were able to observe distinct changes in cell population structure in the synovial fluid of PsA patients compared to the blood. While some populations diminished (i.e. B cells), others expanded, including memory T cells and CD14 myeloid populations. Gene expression analysis of these expanded populations demonstrated multiple significant differences between the blood and synovial fluid that was shared across PsA patients.
Conclusions: We have generated an in-depth map of the immune landscape in PsA blood and matched synovial fluid in combination with a cell-specific transcriptomic analysis of expanded synovial cell populations to reveal novel inflammatory modules in PsA pathogenesis.

P.A3.01.20

BATF Levels in Chronic Lymphocytic Leukemia Patients

M. GELMEZ[1], *S. Cinar*[1], *G. Ozcit*[1], *I. Yonal*[2], *A. Daglar-Aday*[1], *G. Deniz*[1], *M. Aktan*[3];
[1]*Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology,, Istanbul, Turkey,* [2]*Istanbul University, Istanbul Faculty of Medicine, Department of Hematology,, Istanbul, Turkey,* [3]*Istanbul University, Istanbul Faculty of Medicine, Department of Hematology, Istanbul, Turkey.*

Chronic lymphocytic leukemia (CLL) is characterized by accumulation of $CD5^+CD19^+$ cells in the peripheral blood and lymphoid organs. BATF has an essential role in Tfol, Th17 cells and B cell expansion, isotype switching, AID expression and differentiation. The findings show increased AID expression, number of Tfol and Th17 cells in CLL patients, but the role of BATF is not known in CLL pathogenesis. In this study, *BATF* mRNA and protein expressions in CLL were investigated.
Peripheral blood samples were collected from 37 patients with CLL and 16 healthy subjects. Total RNA was isolated from whole blood samples and cDNA synthesis was done. *BATF* and a reference gene, *HPRT1,* were analyzed by real-time PCR. The relative expression levels were calculated by using ΔC_T method. CLL-B ($CD5^+CD19^+$) and Tfol ($CD3^+CD4^+CXCR5^+$) cells were evaluated after staining with anti-CD5, -CD19, -CD3, -CD4, -CXCR5, and intracytoplasmic BATF monoclonal antibodies using flow cytometry according to whole blood lysing protocol.
Compared to healthy controls, *BATF* mRNA levels (p= 0.05); $BATF^+$ and $BATF^+CD19^+$ cells (p= 0.0013 and p< 0.0001, respectively); BATF expression in both $CD19^+$ and $CD3^+$ cells (p=0.0011 and p=0.003, respectively); $CD3^+CD4^+CXCR5^+$ cells (p=0.007) were increased in patients with CLL and also high BATF expression of $CD3^+CD4^+CXCR5^+$ Tfol cells (p<0.001) were found.
Increased BATF expression of different lymphocyte subtypes in patients with CLL was observed. Given the current role of BATF with some molecules associated with the pathogenesis of CLL, our findings provide the impression that BATF could play a role in the biology of CLL.

P.A3.02 Immunomonitoring and biomarkers - Part 2

P.A3.02.01

Complement factor H increased and associated with elevated oxidative stress markers and IL-1β in Algerian Behçet's disease patients.

A. Chekaoui[1], ***H. Belguendouz***[1], *K. Lahmar*[1], *M. Terahi*[2], *F. Z. Mazari*[2], *F. Otmani*[3], *D. Hakem*[4], *C. Touil-Boukoffa*[1];
[1]*Cytokines and NO Synthases team, LBCM, USTHB, Algiers, Algeria,* [2]*Ophtalmology department, CHU Nafissa Hammoud, Algiers, Algeria,* [3]*Internal medicine department, CHU Mustapha Bacha, Algiers, Algeria,* [4]*Internal medicine department, CHU Bab ElOued, Algiers, Algeria.*

Background: Behçet's disease (BD) is a multisystem disease. It stands at the crossroad between autoimmunity and autoinflammatory disorders. In the present study, we aimed to assess the plasma level of complement factor H (CFH) and elucidate its possible correlation with oxidative stress markers and the proinflammatory cytokine IL-1β in Algerian BD patients.
Patients and methods: We investigated the CFH, stress oxidative markers (Nitric oxide (NO), Advanced oxidized proteins products (AOPP)) and IL-1β in Algerian BD patients (78: Active BD patients (ABP, 28) and Inactive BD patients (IBP, 50) referring to disease activity and clinical manifestations compared to healthy controls (HC, 41). Mann–Whitney U and Pearson correlation tests were used for statistical analyses.
Results: CFH levels significantly increased in ABP and IBP (p<0.0001) compared to HC, whereas there is no significant difference (p=0.05) between ABP and IBP. NO and AOPP levels significantly increased in ABP (p<0.001) compared to IBP and HC and in IBP (p<0.001) versus HC. ABP displayed higher plasma levels of IL-1β (p<0.05) versus IBP and HC (P<0.001), also IBP showed higher levels of IL-1β (p<0.01) versus HC.
CFH significantly and positively correlated with AOPP (r=0.414, p=0.0015) and (r=0.5858, p=0.0139) in BD patients and ABP respectively. Moreover, CFH positively correlated with NO (r=0.290, p=0.028) and with IL-1β (r=0.523, p<0.05) in BD patients.
Conclusion: Our study highlights an overexpression of CFH correlated with high levels of oxidative stress markers and IL-1β. We suggest to further study this relationship to illuminate alternative paths of therapeutics in BD.

P.A3.02.02

Analysis of the immune system in patients with hereditary hemochromatosis

V. Bönnemann*, M. Claus, P. Bröde, K. Golka, C. Watzl;*
IfaDo, Dortmund, Germany.

Hereditary Hemochromatosis (HH) is an autosomal recessive disorder of the iron metabolism. The typical systemic iron overload in this disease can cause dysfunction of several organs by iron accumulation. Iron is crucial for cell function, but on the other hand, it produces reactive oxygen species (ROS) by the catalysis of important chemical reactions. Since ROS are known to cause oxidative stress and cellular damage, a precise regulation of iron within cells is necessary. The identification of the HFE gene was a major breakthrough for the understanding of HH. This gene encodes for a novel major histocompatibility complex class 1-related molecule, which play important roles in the immune system. Due to the fact that some HH patients showed aberrant NK cell functionalities in preliminary studies, we wanted to examine whether NK cells could be influenced by iron overload in HH patients.In order to investigate the properties of HH NK cells, PBMC of hemochromatosis patients and age-matched controls were used for immunophenotyping and functional assays such as degranulation and chromium-release assay. In addition, a cytometric bead array and a ferritin ELISA were performed. We observed increased basal and stimulated production of pro-inflammatory cytokines, assuming a distinct functionality of HH PBMC compared to controls. In addition we did not find aberrant NK cell phenotypes, but a general decrease of total granulocyte numbers. These data underline the complexity and sensitivity of the immune system to systemic influences.

P.A3.02.03

The expression profile of the ubiquitin-like modifier FAT10 in immune cells suggests cell type-specific functions

R. Schregle[1,2], *M. M. Mah*[1], *S. Müller*[1], ***F. Brockmann***[1], *A. Aichem*[2], *M. Basler*[1,2], *M. Groettrup*[1,2];
[1]*Division of Immunology, Konstanz, Germany,* [2]*Biotechnology Institute Thurgau, Kreuzlingen, Switzerland.*

The TNF and IFN-γ-inducible ubiquitin-like modifier HLA-F adjacent transcript 10 (FAT10) is most prominently expressed in immunological tissues but information regarding basal expression and inducibility of FAT10 in the different types of immune cells is still lacking. Hence, we investigated FAT10 mRNA expression in the major human and murine immune cell subsets, and FAT10 protein expression in human leukocytes. We isolated the different human leukocytes from peripheral blood and the murine immune cell subsets from spleen. The purified leukocytes were left untreated or stimulated with TNF and INF-γ or LPS to induce FAT10 followed by quantitative real-time PCR or western blot analysis. Basal expression of FAT10 mRNA and protein was generally low but strongly up- regulated by IFN-γ and TNF in all immune cell subsets. LPS treatment induced FAT10 expression marginally in human CD8 + T cells and murine granulocytes, but it increased Fat10 expression significantly in murine regulatory T cells. Yet, in human CD8 + T cells, natural killer cells, natural killer T cells, and dendritic cells, the FAT10 mRNAwas expressed without induction. Similarly, murine macrophages, monocytes, and regulatory T cells expressed Fat10 in the absence of stimulation. In summary, our findings suggest particular functions of FAT10 in these cell types. Furthermore, we observed not only a cell type-specific but also a species-specific basal FAT10 expression profile. Our data will serve as a guideline for future investigations to further elucidate FAT10's role in the immune system.

P.A3.02.04

Personalized monitoring of immune system from undifferentiated arthritis to rheumatoid arthritis in humans and during treatment - a possible practical usage of cytokine profile

E. Bryl[1], *E. Brzustewicz*[1], *I. Bzoma*[1], *M. Bykowska*[2], *M. Szarecka*[2], *A. Daca*[1], *J. M. Witkowski*[3];
[1]*Department of Pathology and Experimental Rheumatology, Medial University of Gdańsk, Gdańsk, Poland,* [2]*Pomeranian Center of Rheumatology, Sopot, Poland,* [3]*Department of Pathophysiology, Medial University of Gdańsk, Gdańsk, Poland.*

Introduction. Undifferentiated arthritis (UA) being usually the first clinical representation of many forms of specific arthritis mostly progresses to rheumatoid arthritis (RA). The immunological studies in established RA showed the profound changes in peripheral blood $CD4^+$ T cells and changed the paradigm of RA being a joint disease only. Our goal was to monitor the cytokine profile in patients progressing from early UA to RA and being monitored during treatment.
Patients and methods: The group of UA patients developing RA (UA→RA) was identified from a total of 121 people with arthralgia. All subjects underwent clinical and laboratory evaluation, including acute phase reactants (APRs) and autoantibodies (anti-CCP, RF, ANA-HEp-2). Cytokines IFN-γ, IL-10, TNF, IL-17A, IL-6, IL-1β, and IL-2 in sera and mononuclear cell supernatants were assayed using BD™ CBA Flex Sets. UA→RA patients were followed up for six months since the final RA diagnosis and DMARDs treatment.
Results: 34.5% of patients with UA developed RA. We observed specific cytokine patterns characterizing each patient, which altered during course of disease. We distinguished three UA→RA cohorts with different cytokine profiles: the group of patients susceptible to the therapy, the group refractory to the therapy and the group with variable responses to the therapy.
Conclusions: The serum cytokine profiles change in the course of RA and may be potentially used for optimization of RA monitoring. The personal profile including multiplexed cytokine patterns in serum and supernatant may be likely utilized for optimization of therapy introduction and monitoring.

P.A3.02.05

T_{FH}2 and T_{FH}17-Polarized Cells Contribute Highly to Circulating Follicular Help T Cell Response following Measles, Mumps, and Rubella (MMR) Vaccination

G. Elias[1], *E. Bartholomeus*[2], *P. Meysman*[3], *N. De Neuter*[3], *A. Suls*[2], *N. Keersmaekers*[1], *H. Jansens*[4], *S. van der Heijden*[1], *E. Smits*[1], *N. Hens*[1], *K. Laukens*[3], *P. Beutels*[1], *P. Van Damme*[1], *G. Mortier*[2], *V. Van Tendeloo*[1], *B. Ogunjimi*[1];

[1]*Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Belgium,* [2]*Centre of Medical Genetics, University of Antwerp, Antwerp, Belgium,* [3]*Advanced Database Research and Modelling (ADReM), Department of Mathematics and Computer Science, University of Antwerp, Antwerp, Belgium,* [4]*Department of Microbiology , Antwerp University Hospital, Antwerp, Belgium.*

The sequence of immunological pathways that cooperate in the successful induction of protective antibody response is not completely understood in humans. T follicular helper (T_{FH}) cells provide help to B cells in germinal centres, in which class switching, affinity maturation, and generation of long-lived plasma cells and memory B cells occur. However, studying T_{FH} in humans is difficult since secondary lymphoid tissues are only accessible through invasive techniques.
Recently, circulatory $CXCR5^+$ $CD4^+$ T cells have been shown to represent memory counterparts of bona fide T_{FH} cells in peripheral blood. Circulatory T_{FH} (cT_{FH}) were further shown to be constituted of three major subsets based on the expression of two chemokine receptors, CXCR3 and CCR6. In this paradigm, T_{FH}2 and T_{FH}17- polarized responses ($CXCR3^-$ $CCR6^-$ and $CXCR3^-CCR6^+$, respectively) proved to be superior to T_{FH}1- polarized responses ($CXCR3^+CCR6^-$) in helping B cells. Interestingly, in longitudinal studies following influenza vaccination, activated cT_{FH} were shown to be T_{FH}1-polarized but not T_{FH}2 or T_{FH}17-polarized. Here, we show that following administration of a measles/mumps/rubella (MMR) vaccine, cT_{FH} are more diversely polarized, in which T_{FH}2 and T_{FH}17-polarized responses accompany the inferior T_{FH}1-polarized response. We observed a peak of cT_{FH} activation at day 3, decreasing at day 7 and returning to the initial level of activation at day 21. These data provide insights into the cT_{FH} cell biology and dynamics and further support the notion that vaccine strategies that promote superior cT_{FH} cell responses may improve vaccine efficacy.

P.A3.02.06

Tracking dye-independent approach to identify and sort viable antigen-specific T cells

G. Elias*, B. Ogunjimi, V. Van Tendeloo;*
Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Belgium.

Proliferation after antigen encounter is a key feature of T cells contributing to the robust recall immune responses that form the basis of vaccination. Proliferation assays are used to study T cell function and for immune monitoring. Proliferative capacity of T cells is routinely assessed *in vitro* using tracking dyes such as carboxyfluorescein succinimidyl ester (CFSE) or through detecting intracellular upregulation of the nuclear protein, Ki-67. However, labelling with tracking dyes is cumbersome, toxic to cells and demands extensive washing, resulting in a substantial loss of cells, while nuclear Ki-67 staining cannot be used when sorting viable cells, thereby limiting its applicability.
Here we introduce a flow cytometric technique for identification of proliferating T cells that is dye-independent and allows for sorting of viable cells. CD71, a transferrin receptor, and CD98, an amino acid transporter, have been described as surface markers of T-cell activation. We show here that CD71 and CD98 upregulation plateaued 24 and 48 hours after stimulation, respectively, and that CD71 expression peaked on the more recent progeny T cells while CD98 upregulation was more stable on dividing cells up to 6 days of expansion. We further demonstrate that αβ T cells and γδ T cells bearing upregulated expression of CD71 and CD98 were also $CFSE_{low}$ and Ki-67^{high} T cells.
In conclusion, we show that CD71 and CD98 can be used to identify and sort viable antigen-specific T cells following *in vitro* expansion and provides a valid alternative to assays based on tracking dyes or Ki-67 upregulation.

P.A3.02.07

The measurement of IP-10 release in the Bovigam PC-EC and PC-HP assays for the optimal diagnosis of Bovine tuberculosis in African buffaloes (*Syncerus caffer*).

W. J. Goosen*;*
Stellenbosch University, Cape Town, South Africa.

Background: African buffaloes (*Syncerus caffer*) are a maintenance host of *Mycobacterium bovis*, the cause of bovine tuberculosis (bTB). The control of bTB in buffaloes relies on use of the single intradermal comparative tuberculin test (SICTT) and interferon gamma (IFN-γ) release assays (IGRAs) such as commercial Bovigam assays. However, the diagnostic sensitivities of these IGRAs are still believed to be sub-optimal. We aimed to evaluate the diagnostic utility of measuring antigen-dependent interferon gamma-induced protein 10 (IP-10) release as an alternative to measuring IFN-γ levels.
Materials and Methods: *M. bovis*-exposed buffaloes were tested using the SICTT and Bovigam assays, i.e. purified protein derivative (PPD) assay, PC-EC assay and PC-HP assay, and a modification of the PPD assay. Animals positive on any IGRA were slaughtered (n = 63) and classified as *M. bovis*-infected to culture (n = 35) or both culture and bTB pathological findings (n = 41). The relative sensitivity of the SICTT, each IGRA and IP-10 release assay were calculated as the proportion of *M. bovis*-infected testing positive and the diagnostic agreement between assays.
Results: Confirmed *M. bovis*-infected animals: the IP-10(HP) assay showed greatest test sensitivity and the best agreement with the IP-10(EC) assay. The Bovigam PPD assay in combination with either the IP-10(HP)- or the IP-10(EC) assay resulted in the optimal diagnostic sensitivity for both cohorts.
Conclusion: The IP-10(HP) assay had the highest individual relative sensitivity and combining the Bovigam PPD assay with any IP-10 assay produced an optimal combination of blood tests that successfully detected all *M. bovis*-infected animals.

P.A3.02.08

Sample aging profoundly reduces monocyte-, but not T-cell-driven innate immune responses in human whole blood cultures

W. Grievink*, M. Moerland;*
Centre for Human Drug Research, Leiden, Netherlands.

Human whole blood cultures are widely used for investigation of physiological pathways and drug effects *in vitro*. Detailed information on the effect of 'sample aging' (the time-span between blood collection and experimental start) on the experimental outcome is not readily available in the public domain. We studied the effect of sample aging on the ability of immune cells to respond to cell-specific immune triggers (LPS, PMA/ionomycin, SEB). Sample aging at room temperature profoundly inhibited the LPS-induced monocytic cytokine release in minimally diluted whole blood cultures. The reduction ranged from 20-50% after 30 minutes to 80-100% after 10 hours, and differed between cytokines (IL-1β, IL-2, IL-6, IFNγ, TNFα). Sample storage at 4ºC or 37ºC even worsened this. In contrast, PMA/ionomycin- and SEB-induced cytokine release, both mainly T-cell-driven, remained much more stable during sample storage. Intracellular cytokine staining revealed that the number of LPS-responding cells was not impacted by sample aging, and reduced LPS responsivity could also not be explained by apoptosis or downregulated TLR4 expression. Addition of culture medium during aging did not result in a more stable monocyte response to LPS during aging. Thus, we speculate that sample aging induces an inhibitory pathway downstream from TLR4 in monocytes. These results underline the importance of quick sample handling when investigating innate immune responses in whole blood, especially for monocyte responses.

P.A3.02.09

IL-7 induces an epitope masking of common gamma chain protein in IL-7 receptor signaling complex

Y. Jo, B. Lee, G. Kim, ***C. Hong****;*
Pusan National University, Yangsan, Korea, Republic of.

IL-7 signaling via IL-7Rα and common γ-chain (γc) is necessary for the development and homeostasis of T cells. Although the delicate mechanism in which IL-7Rα downregulation allows the homeostasis of T cell with limited IL-7 has been well known, the exact mechanism behind the interaction between IL-7Rα and γc in the absence or presence of IL-7 remains unclear. Additionally, we are still uncertain as to how only IL-7Rα is separately downregulated by the binding of IL-7 from their IL-7Rα/γc complex. We demonstrate here that 4G3, TUGm2, and 3E12 epitope masking of γc protein is induced in the presence of IL-7, indicating that the epitope alteration is induced by IL-7 binding to the preassembled receptor core. Moreover, the epitope masking of γc protein is inversely correlated with the expression of IL-7Rα upon IL-7 binding, implying that the structural alteration of γc might be involved in the regulation of IL-7Rα expression. The conformational change in γc upon IL-7 binding may not contribute only to form the functional IL-7 signaling complex but also to optimally regulate the expression of IL-7Rα.

P.A3.02.10

Fecal galectin-3 - new promising biomarker for severity and progression of colorectal carcinoma

M. Jurisevic*, N. Gajovic, N. Zdravkovic, M. Jovanovic, M. Jovanovic, D. Vojvodic, I. Jovanovic;*
Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Serbia, Kragujevac, Serbia.

The aim of study was to determine systemic and fecal values of Galectin-3, pro- and anti-inflammatory cytokines, in patients with CRC and the relationship with clinicopathological aspects. Concentrations of Galectin-3, TNF-α, TGF-β, IL-10 and IL-1β were analyzed in samples of blood and stool of 60 patients with CRC. Systemic concentration of TNF-α was significantly lower in patients with severe disease (advanced TNM stage, nuclear grade and poor histological differentiation) as in patients with more progressive CRC (lymph and blood vessels invasion, presence of metastasis). Fecal values of anti-inflammatory cytokines TGF-β and IL-10 were increased in patients with severe stadium of CRC.

Fecal concentration of Gal-3 was enhanced in CRC patients with higher nuclear grade, poor tumor tissue differentiation, advanced TNM stage and metastatic disease. Gal-3/TNF-α ratio in sera and feces had a higher trend in patients with severe and advanced disease. Positive correlation between fecal Gal-3 and disease severity, tumor progression and biomarkers AFP and CEA, respectively was also observed. Predomination of Gal-3 in patients with advanced disease may implicate on its role in limiting ongoing proinflammatory processes. The fecal values of Gal-3 can be used as valuable marker for CRC severity and progression.

P.A3.02.12

Complement component C1q as serum biomarker to detect active tuberculosis

R. Lubbers[1], *J. Sutherland*[2], *D. Goletti*[3], *F. A. Verreck*[4], *A. Geluk*[1], *T. H. Ottenhoff*[1], *S. A. Joosten*[1], *L. A. Trouw*[1];
[1]*Leiden University Medical Center, Leiden, Netherlands,* [2]*Medical Research Council Unit, Banjul, Gambia,* [3]*National Institute for Infectious Diseases, Rome, Italy,* [4]*Biomedical Primate Research Centre, Rijswijk, Netherlands.*

Introduction: Tuberculosis (TB) remains a major threat to global health. Currently, diagnosis of active TB is hampered by the lack of specific biomarkers that discriminate active TB from other (lung) diseases or latent TB infection (LTBI). The complement system is an important part of the innate immune system and integrated gene expression analyses have revealed that complement genes, in particular the C1q genes, were expressed at higher levels in active TB compared to LTBI.
Methods: C1q protein levels were determined using ELISA in sera from patients, from geographically distinct populations, with active TB, LTBI as well as disease controls.
Results: Serum levels of C1q were increased in active TB compared to LTBI in four independent cohorts and discriminated with an AUC of 0.77 [0.70 ; 0.83]. After six months of TB treatment, levels of C1q had normalized to those of endemic controls, indicating an association with disease rather than individual genetic predisposition. Importantly, C1q levels in sera of TB patients were significantly higher as compared to patients with sarcoidosis or pneumonia, clinically differential diagnoses. Moreover, exposure to other mycobacteria such as *M. leprae* (leprosy patients) or BCG (vaccinees) did not present with elevated levels of serum C1q. In agreement with the human data, in non-human primates challenged with *Mycobacterium tuberculosis*, increased serum C1q levels were detected in animals that developed progressive disease.
Conclusions: Circulating C1q is a novel TB biomarker, which discriminates active TB from most other conditions, including other lung diseases, and could have added value in diagnosing TB.

P.A3.02.13

Cellular immune response to T3SS proteins in humans vaccinated with live bacterial vaccine

A. M. Lyapina[1], *V. A. Feodorova*[1,2], *S. S. Zaitsev*[1], *M. A. Khizhnyakova*[1,2], *L. V. Sayapina*[3], *M. V. Telepnev*[4], *O. V. Ulianova*[1], *E. P. Lyapina*[5], *S. S. Ulyanov*[1,6], *V. L. Motin*[4];
[1]*Laboratory for Molecular Biology and NanoBiotechnology, Federal Research Center for Virology and Microbiology (FRCViM), Branch in Saratov, Saratov, Russian Federation,* [2]*Department for Microbiology, Biotechnology and Chemistry, Saratov State Agrarian University named after N.I. Vavilov, Saratov, Russian Federation,* [3]*Department of Vaccine Control, Scientific Center on Expertise of Medical Application Products, Moscow, Russian Federation,* [4]*Department of Pathology, Department of Microbiology & Immunology, University of Texas Medical Branch, Galveston, United States,* [5]*Department for Infectious Diseases, Saratov State Medical University named after V.I. Razumovsky, Saratov, Russian Federation,* [6]*Department for Medical Optics, Saratov State University, Saratov, Russian Federation.*

Type III Secretion System (T3SS) is a special “nanomachine” widely used by Gram-negative bacteria for the delivery of effector proteins to mammalian target cells. In fact, T3SS components may participate in eliciting effective adaptive response. In this study we investigated human cellular immune response to the live plague vaccine (LPV), an attenuated *Y. pestis* EV strain line NIIEG possessing T3SS. Highly pure T3SS recombinant proteins, LcrV and YopM, were used for the *in vitro* stimulation of PBMCs collected from multiply immunized donors (n=18) and naïve control individuals (n=6) to assess lymphocyte proliferation and Th1/Th2/Th17 polarization. We found that although there was no significant difference between vaccinated and control groups in proliferative responses to all antigens, re-stimulation with YopM induced a high proliferative reaction of lymphocytes from vaccinees exceeding those with control F1 antigen ($p>0.05$) and LcrV ($p<0.001$) 2.1-fold and 3.3-fold respectively. Marked proliferative response to YopM was accompanied by high production of IFN-γ and TNF-α, which was specific for vaccinees ($p<0.05$). On the contrary, F1 stimulus specifically induced mixed Th1/Th2 response with IFN-γ, TNF-α and IL-4 secretion in vaccinees ($p<0.01$). *In vitro* stimulation with LcrV induced an increased secretion of IL-10 exclusively in vaccinated persons ($p<0.05$), while the similar response to YopM and F1 was nonspecific ($p>0.05$). In conclusion, we have shown that T3SS effector protein YopM, but not LcrV, is likely to be strongly involved in the cellular response elicited by LPV in humans. This antigen-specific response was Th1-polarized. This study was supported by the RFBR #18-016-00159.

P.A3.02.14

IL-10 and IL-17 chronic low grade inflammation marker in colorectal cancer patients and healthy elderly

I. Pantsulaia[1], *A. Aladashvili*[1], *M. Iobadze*[1], *N. Kikodze*[1], *T. Atamashvili*[1], *T. Chikovani*[2];
[1]*Vl.Bakhutashvili Institute of Medical Biotechnology, Tbilisi State Medical University, Tbilisi, Georgia,* [2]*Tbilisi State Medical University, Tbilisi, Georgia.*

Background: Permanent exposure of environmental factors and numerous interactions with pathogen leads to a chronic inflammatory state in older individuals. In fact, acute inflammation is a beneficial process but with ageing it becomes chronic and leads to tissues dysfunction or degeneration. Recently, many studies have been demonstrated that chronic inflammation is associated with all stages of cancer development increasing its risk, supporting and promoting cancer progression. Moreover preventive treatment with anti-inflammatory drugs like aspirin reduces the incidence and mortality for colorectal cancer. Consequently, evaluation inflammatory state in different tumors and age matched individuals is essential for designing new personalized treatments. **Aim:** The main aim of our study was to evaluate the alterations of cytokine (IL-17, IL-10, IL-22, TNF-a, IL-6, IL-4 and IFN gamma) plasma levels in colorectal (n=50) and elderly population (n=150). Plasma specimens from studied individuals were obtained in the morning after fasting. The samples were analyzed using ELISA kits. **Results:** The IL-10, IL-17, IL-22 levels was higher in colorectal cancer patients than healthy individuals. IL-10 concentrations were significantly ($P<0.01$) decreased in elderly group comparing to young people. On the contrary to IL-10, IL-17 levels were higher in aged persons than young population. The correlations among studied cytokines were strong and significant in both groups. **Conclusion:** The outcomes of study suggest a shift the inflammatory status in elderly population, however, could not prove a clear and strong polarization. The ratio of IL-10/IL-17 concentrations should be used as indicator of declining health in aged individuals.

P.A3.02.15

Utility of serum levels of interleukin 13 in patients with sarcoidosis.

P. Sohal[1], *K. Upadhyay*[1], *A. Ali*[1], *H. Aggarwal*[2], *M. Goel*[1], *V. Singh*[3], *K. Madan*[1], *A. Mohan*[1], *R. Guleria*[1];
[1]*Pulmonary Medicine and Sleep Disorders, AIIMS, New delhi, India,* [2]*Biochemistry, AIIMS, New delhi, India,* [3]*Biostatistics, AIIMS, New delhi, India.*

Introduction: Cytokines are inflammatory mediators which play an important part in immunopathognesis of sarcoidosis. Cytokine IL-13 produced by type 2 helper T cells (TH2), mast cells, eosinophils and basophils, is the key mediator during effector phase of allergic inflammation. Our study aimed to describe the variations of serum levels of IL-13 among patients with sarcoidosis and healthy controls. **Methods:** In this study 57 newly diagnosed sarcoidosis patients and 32 healthy controls were enrolled. A total of 27 sarcoidosis patients were followed up for six months after initiation of treatment. Serum levels of IL-13 were measured using Enzyme-linked Immunosorbent Assay (ELISA) among sarcoidosis patients at baseline, post six months of treatment and healthy controls. **Results:** Median (range) of serum IL-13 levels in patients with sarcoidosis at baseline, after treatment and in healthy controls were 16.12 (7.289-93.90)pg/ml, 14.81 (7.77-29.51)pg/ml and 11.59 (4.265-31.24) pg/ml respectively. The IL-13 levels were higher among patients at baseline compared to healthy controls ($p=0.01$). IL-13 levels were significantly reduced among patients after six months of treatment as compared to baseline ($p=0.03$). There was no significant difference in IL-13 levels after follow up when compared to healthy controls. **Conclusion:** The data shows the involvement of IL-13 in inflammation of sarcoidosis and can be a useful marker of disease activity. Serum IL- 13 levels at baseline may significantly aid in diagnosis of sarcoidosis. To confirm this, large population study is warranted.

P.A3.02.16

Amniotic fluid complement component C3 and complement factor I levels in pregnancies complicated by the preterm prelabor rupture of membranes

O. Soucek[1], *I. Musilova*[2], *C. Andrys*[1], *J. Krejsek*[1], *M. Kacerovsky*[2,3];
[1]*Department of Clinical Immunology and Allergy, University Hospital, Hradec Kralove, Czech Republic,* [2]*Department of Obstetrics and Gynecology, University Hospital, Hradec Kralove, Czech Republic,* [3]*Biomedical Research Center, University Hospital, Hradec Kralove, Czech Republic.*

Introduction: Preterm prelabor rupture of membranes (PPROM) is characterized by the rupture of fetal membranes and leakage of amniotic fluid before onset of regular labor activity prior to gestational age 37 weeks. It is a significant public health problem because it complicates about 3% of all pregnancies (about one-half of spontaneous preterm deliveries) and has a significant health, social and economic impact on society. Etiology of PPROM is not yet fully elucidated, but it is known that is commonly accompanied by microbial invasion of the amniotic cavity (MIAC) and/or intra-amniotic inflammation (IAI). **Objective:** The aim of this study was to determine the amniotic fluid complement component C3 and complement factor I concentrations in women with PPROM based on MIAC, IAI and microbial-associated IAI. **Methods**: One hundred fifty-nine women with singleton pregnancies complicated by PPROM were included in the study. Amniotic fluid samples were obtained by transabdominal amniocentesis and were assayed for complement component C3 and complement factor I concentrations by ELISA tests. **Conclusion:** The presence of IAI and microbial-associated IAI is connected with higher concentrations of complement component C3 and lower concentrations of complement factor I. MIAC alone had no impact on C3 or complement factor I levels in amniotic fluid. The results show that complement may be involved in etiology of PPROM. **Acknowledgments:** This work was supported by the Faculty Hospital in Hradec Kralove and by Charles University in Prague, Faculty of Medicine in Hradec Kralove, Czech Republic, project “PROGRES Q40/10”

P.A3.02.17

Serum levels of BAFF and IL17 induces tissue damage in pauci immune small vessel vasculits and glomerulonephritis

M. Stangou[1], *A. Fylaktou*[2], *D. Daikidou*[1], *C. Nikolaidou*[3], *C. Stamos*[4], *E. Sampani*[1], *E. Moraiti*[1], *A. Papagianni*[1];
[1]*Department of Nephrology, Aristotle University of Thessaloniki, Thessaloniki, Greece,* [2]*Department of Immunology, National Peripheral Histocompatibility Center, Hippokration Hospital, Thessaloniki, Greece,* [3]*Department of Pathology, Thessaloniki, Greece,* [4]*Department of Pathology, General University Hospital of Alexandroupolis, Alexandroupolis, Greece.*

Introduction: Renal impairment in small vessel vasculitis (SVV) is characterised by inflammation, proliferation and necrosis. B cell activating or B lymphocyte stimulating factor (BAFF/BLyS) and Interleukin 17 (IL-17) may be involved in the pathogenesis of tissue damage. **Patients-Methods.** BAFF and IL17 levels were measured by ELISA, in serum from 60patients with SVV and glomerulonephritis [33 female, age 57.7yrs (25-80)] collected at the day of renal biopsy, before any treatment was applied, and results were correlated with renal pathology and urinary excretion of INF-γ, IL-10 and G-CSF. Treatment protocol consisted of prednisolone+cyclophosphamide as induction, with or without plasmapheresis, followed by prednisolone+azathioprine for at least 2yrs. Follow up of the patients was 28.8±30months. **Results.** Serum levels of BAFF and IL-17 were significantly increased in SVV patients compared to controls (1601.45±1.34 vs. 960.33±305.24mg/dl, p=0.001 and 21.7±25.9 vs. 12.4±3.8mg/dl, p=0.009 respectively). Both serum BAFF and IL-17 levels were significantly increased in ANCA(+) compared to ANCA(-) patients (1663.5±1309mg/dl vs.1073.8±521.8mg/dl, p=0.02 and 24.9±30mg/dl vs. 15.2±2.8mg/dl, p=0.05, respectively). Serum BAFF levels correlated significantly with urinary IL-10 (p=0.04) and INF-γ (p=0.03), percentage of cellular crescents (p=0.04), while serum IL-17 was correlated with urinary IL-17 levels (p=0.04), degree of interstitial fibrosis (p=0.04) and renal function outcome (p=0.02). **Conclusions.** BAFF and IL-17 are increased in the serum of patients with glomerulonephritis secondary to SVV, especially in the presence of ANCA(+). BAFF at early stages may be implicated in inflammation, through the production of IL-10 and INF-γ, while IL-17 in more advanced stages, induces fibrosis and deterioration of renal function.

P.A3.02.18

Epidermal Growth Factor (EGF) as biomarker of renal function outcome in different forms of primary glomerular diseases.

M. Stangou[1], *A. Fylaktou*[2], *E. Sampani*[1], *C. Nikolaidou*[3], *C. Stamos*[4], *D. Daikidou*[1], *E. Moraiti*[1], *A. Papagianni*[1];
[1]*Department of Nephrology, Aristotle University of Thessaloniki, Thessaloniki, Greece,* [2]*Department of Immunology, National Peripheral Histocompatibility Center, Hippokration Hospital, Thessaloniki, Greece,* [3]*Department of Pathology, Hippokration Hospital, Thessaloniki, Greece,* [4]*Department of Pathology, General University Hospital of Alexandroupolis, Thessaloniki, Greece.*

Introduction and aims: EGF acts through EGF receptor and facilitates regeneration of tubular epithelial cells after injury. We investigated the role of urinary EGF excretion as biomarker for pathology and renal function outcome in glomerulonephritis.
Msthods: EGF urinary levels were estimated in 3 forms of glomerular diseases: (1) IgA nephropathy (IgAN), as chronic glomerulonephritis, [n=50, 21female, age 39.8yrs(18-65)], (2) pauci immune rapidly progressive glomerulonephritis (RPGN), as acute glomerulonephritis, [n=38, 17 female, age 59.5yrs(25-80)] and, (3) Nephrotic syndrome (NS) due to focal segmental glomerulosclerosis (FSGS) [n=23, 9 female, age 47.5yrs(19-79)] and minimal change disease (MCD) [n=12, 7female, age 45.5yrs(37-62)]. Ten healthy volunteers were used as controls. First morning urine samples were collected on the day of renal biopsy. Patients were followed up for 7.5±2.1yrs.
Results: EGF urinary levels were: IgAN 0.13±0.2, FSGS 0.19±0.2, MCD 0.7±0.4, RPGN 0.15±0.3, controls 0.14±0.07pg/mgUcr. EGF urinary levels had significant negative correlation with severity of interstitial fibrosis, r=-0.6, p=0.02, in IgAN, with the percentage of fibrous crescents, r=-0.6, p=0.01 in RPGN, and finally, with the percentage of global sclerosed glomeruli, r=-0,5, p=0,04, degree of fibrosis, r=-0.6, p=0.005 and interstitial infiltration, r=-0.6, p=0.01 in patients with NS.
Declining renal function was associated with reduced urinary EGF levels in IgAN (0.04±0.04 vs. 0.2±0.2pg/mgUcr, p=0.01), and FSGS (0.007±0.004 vs. 0.6±0.04pg/mgUcr, p=0.009), but not in RPGN (0.05±0.1 vs. 0.2±0.4pg/mg cr, p=NS).
Conclusions: Reduction of EGF urinary levels in patients with glomerulonephritis is associated with "chronic" histological changes, and can predict renal function outcome mainly in patients with chronic forms of glomerulonephrits.

P.A3.02.19

Circulating cytokines predict response to anti-PD1 therapy in NSCLC

I. Zizzari, *M. Dionisi, A. Botticelli, A. Di Filippo, F. Di Pietro, C. Scopelliti, A. Ugolini, C. Napoletano, A. Rughetti, P. Marchetti, M. Nuti;
Sapienza University of Rome, Rome, Italy.*

Background: Despite the recent successes of immunotherapy in the treatment of non small cell lung cancer (NSCLC), only 20-30% of patients have a long-term benefit from immunotherapy, while the remaining 70-80% result resistant. The identification of responder patients represents the open issue of immunoncology. In the context of several biomarkers the study of cytokine profile represents a promising approach. Chemokines, such as CXCL10 and sICAM, can facilitate chemotactic recruitment of TILs thus favouring their intratumoral trafficking accumulation and increasing the immune response against tumor. In the present study, we explored the prognostic impact of circulating cytokines in 18 NSCLC patients undergoing anti-PD-1 treatment.**Methods**: Sera from 18 NSCLC patients in treatment with nivolumab, were analysed using the ProcartaPlex Human Inflammation Panel. Samples were measured by BioPlex Magpix Multiplex Reader and data analysis was performed using Bioplex Manager MP software.**Results:** Seven out of 18 patients presented early progression, defined as progression of disease within 6 months from the beginning of nivolumab treatment. The median value of CXCL10 and sICAM in early progressor patients were 1339 pg/ml and 255202 pg/ml respectively, while in no progressor patients were 2334 pg/ml for CXCL10 and 370000 pg/ml for sICAM. We found a significant association between the circulating levels of the chemokine CXCL10 and sICAM and early progression (p<0.05).**Conclusions:** CXCL10 and sICAM seem to be associated with response to immunotherapy and could predict resistance to anti-PD-1 treatment. These preliminary results suggest the possibility of design and select alternative strategies to overcome the resistance in progressor patients

P.A3.02.20

Methods for whole blood immunomonitoring of clinical samples for flow and mass cytometry studies.

P. Rybakowska[1], *C. Burbano*[1], *R. Aguilar Quesada*[2], *C. Marañón*[1], *M. E. Alarcón Riquelme*[1,3];
[1]*Genyo. Centre for Genomics and Oncological Research: Pfizer / University of Granada / Andalusian Reg, Granada, Spain,* [2]*Biobanco del Sistema Sanitario Público de Andalucía, Andalusian Public Health System Biobank, Granada, Spain,* [3]*Institute for Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.*

The multicentric PRECISESADS project aims at the molecular re-classification of the systemic autoimmune diseases combining molecular and cellular -omics, including flow and mass cytometry. Therefore to avoid center bias and technical variations we considered the preservation of collected samples until the time of analysis. Herein we compare two buffers for whole blood fixation and cryopreservation: Smart Tube Proteomic Stabilizer (PROT1) and BD phosphoflow lyse/fix buffer (BD). Eight surface markers were verified using flow cytometry, and 15 surface and 8 intracellular markers were verified for mass cytometry upon R848 stimulation in fresh blood, and compared with the results obtained after fixation and freezing. The verified storage time was 1 and 6 months for flow and 1 month for mass cytometry. Our data show that whole blood can be preserved and stored until acquisition; however care needs to be taken when designing the antibody panel. In flow cytometry BD buffer allows monocyte/granulocyte discrimination based on FSC/SSC, while with PROT1 granulocytes or monocytes marker is needed. In both technologies similar percentages of major leucocytes and their subpopulations were observed when comparing the two fixation methods to the unfixed condition. A cytokine response study can be done using both buffers, as we did not observe significant differences between the two products. We consider blood preservation as a good method for immunomonitoring of samples that minimizes center bias and technical variation in both flow and mass cytometry studies. Financial support: IMI-JU, GA [115565], with financial contribution from the EU FP7, EFPIA & SANOFI.

P.A3.02.21

Maximizing human immune monitoring studies with mass cytometry

C. Loh[1], *T. Selvanantham*[1], *L. Fung*[1], *M. P. Poulin*[1], *C. B. Bagwell*[2], *M. Inokuma*[2], ***C. E. Rogers***[1], *S. T. Lott*[1];
[1]*Fluidigm, South San Francisco, United States,* [2]*Verity Software House, Topsham, United States.*

Immune monitoring is an essential method for quantifying changes in immune cell populations in chronic inflammation, infectious disease, autoimmune disease and cancer studies. The extreme heterogeneity of immune cells demands a high-parameter approach to more fully and efficiently quantify the immune response in health and disease. Mass cytometry is an ideal solution, enabling the simultaneous detection of over 40 phenotypic and functional markers in a single tube of sample. We report development of a 29-marker panel for mass cytometry based on the Human ImmunoPhenotyping Consortium (HIPC) consensus panel [Maecker et al. *Nature Reviews Immunology* (2012)], expanded to allow identification of additional leukocyte subsets, particularly T cells. Automated data analysis with Verity Software House GemStone™ software has been developed specifically for data collected with the panel. Extensive panel testing for repeatability, reproducibility and agreement of full versus partial panel population identification was performed. Repeatability was tested with a single PBMC sample stained by a single technician in two technical replicates and acquired in triplicate on two Helios™ mass cytometers. SDs for percent of parent were 1% or less for 16 identified populations. Reproducibility was tested by determining the variability in measurements of five PBMC lots stained by five technicians and collected on two Helios instruments. CVs on mean percent of 13 populations were under 15% for all but three of 130 measurements. Lastly, R^2 values for agreement of percent parent populations using the full 29marker panel compared to a 10marker panel for T cell populations were 0.94 or higher.

P.A3.02.22

In depth analysis of antibody reactivities directed against numerous citrullinated peptides derived from the human proteome

H. Thiesen[1], *F. Steinbeck*[2], *E. Schade*[2], *S. Drynda*[3], *J. Kekow*[4];
[1]*Institute of Immunology, Rostock, Germany,* [2]*Gesellschaft für Individualisierte Medizin, Rostock, Germany,* [3]*Helios Fachklinik Vogelsang-Gommern, Gommerrn, Germany,* [4]*Helios Fachklinik Vogelsang-Gommern, Gommern, Germany.*

Introduction: Our analysis is driven by the interest to select citrullinated peptides whose sequences are derived from the human proteome. Panels of peptides were determined that show differential epitope-antibody-reactivities (EAR) as initially found in intravenous immunglobulin preparations, see PMID: 27059896 and PMID: 24244326. Method: Highdensity peptide microarrays representing pairs of peptides were incubated with sera of patients suffering from CCP positive versus CCP negative rheumatoid arthritis (RA). The paired peptides carry either an arginine, whereas the other the same sequence but the arginine replaced by a citrulline The most informative citrullinated peptides were then comparatively evaluated by applying MSD multi-array analysis. Ten different peptides placed per well were either incubated with sera of blood donors or of substratified RA patients. Results: In total, 96 different citrulinated peptides were finally characterized of which 63 were further studied in multi-array analysis, leading to distinct subsets of human derived citrullinated peptides. One peptide panel has the potential to replace the commerical CCP assay, the other panel stratifies CCP negative RA patients and the third one is suitable to determine putative preClinical RA within blood donors. Conclusion: Our comparative computational analysis of the most informative citrullinated peptides specifies proteins that play major roles in conducting and elicitating immune responses as well as in performing cellular functions of intercellular communication. Our data set represent putative candicate peptides whose antibody reactivity profiles might have great values in determining clinical features being causative in initiation and progression of CCP positive RA disease.

P.A3.03 Immunomonitoring and biomarkers - Part 3

P.A3.03.01

The IgG4:IgG RNA ratio differentiates active disease from remission in granulomatosis with polyangiitis: A new disease activity marker?

A. Al-Soudi[1], *M. Doorenspleet*[1], *R. Esveldt*[1], *L. Burgemeister*[1], *L. Hak*[1], *B. Van den Born*[2], *S. Tas*[1], *R. van Vollenhoven*[1], *P. Klarenbeek*[1], *N. de Vries*[1];
[1]*Amsterdam Rheumatology & Immunology Center, Amsterdam, Netherlands,* [2]*Academic Medical Center, Amsterdam, Netherlands.*

Objectives: An important limitation in Granulomatosis with Polyangiitis (GPA) is the lack of disease activity markers. Immunoglobulin G4 positive (IgG4+) B-cells and plasma cells are implicated in the pathogenesis of GPA. We hypothesized that the presence of these cells in peripheral blood could serve as biomarker in GPA.
Methods: We included 32 PR3-ANCA positive GPA patients in a cross-sectional study. Active disease was defined as BVAS≥3 (n=14), remission as BVAS of 0 (n=15) and low disease activity (LDA) as BVAS 1-2 and clinical remission (n=3). Healthy subjects (n=10), patients with systemic lupus erythematosus (n=24), and patients with rheumatoid arthritis (n=19) functioned as controls. An additional longitudinal study was performed in 9 GPA patients. Using a validated qPCR test, we measured the IgG4:IgG RNA ratio in all groups and compared the results to known biomarkers.
Results: The median qPCR score was higher in active GPA (20.7; IQR 12.1-29.3) compared with remission/LDA (3.8; IQR 1.9-5.8) (Mann-Whitney U, p<0.0001) and outperformed other known disease activity parameters in detecting activity. A cut-off qPCR score of 11.2% differentiated active disease from remission/LDA accurately (AUC 0.992). The qPCR test correlated well with the BVAS (Spearman r=0.75, p<0.0001). In the longitudinal study, a decrease in BVAS was associated with a qPCR score reduction.
Conclusions: The IgG4:IgG RNA ratio in GPA accurately distinguishes active disease from remission and correlates well with disease activity in these single-center studies. If these results are confirmed in larger and longitudinal studies, this test might help steering treatment decisions in GPA patients.

P.A3.03.02

Research of Anti-GAD and Anti-IA2 autoantibodies by ELISA test in a series of Moroccan pediatric patient with diabetes type 1

O. BELHIBA[1,2], *B. Ahmed Aziz*[3,4], *F. Jennane*[2,3];
[1]*Faculty of Medicine and Pharmacy Hassan II University- Casablanca, Morocco., Casablanca, Morocco,* [2]*Pediatric Endocrinology Unit, Hopital d'Enfant Abderrahim Harouchi Chu Ibn Rochd, Casablanca, Morocco., Casablanca, Morocco,* [3]*Laboratory of Clinical Immunology, Allergy and Inflammation LICIA, Faculty of Medicine and Pharmacy Hassan II University-Casablanca, Morocco., Casablanca, Morocco,* [4]*Clinical Immunology Unit, Infectious Department, Hopital d'Enfant Abderrahim Harouchi, CHU Ibn Rochd, Morocco., Casablanca, Morocco.*

Introduction : Type I diabetes (T1D) is an autoimmune disease with a asymptomatic period characterized by the destruction of insulin-producing β cells. This preclinical phase is of a variable duration during which various autoantibodies are generated against several beta cell antigens such as : Anti Glutamate Acid Decarboxylase (Anti-GAD), Anti Tyrosine Phosphatase (Anti-IA2). **Objectives :** In this work, we want to evaluate the diagnostic value of Anti-GAD and Anti-IA2 antibodies in a series based on 78 Moroccan subjects initially under 16, suspected T1D. **Patients and Methods :** Our study concerns 78 children aged from 1 to 16 years followed for an evocative table of T1D. Samples of patients were analyzed by ELISA tests using Anti GAD and Anti-IA2 commercial kits (EUROIMMUN). **Results and Discussion :** Our series consists mainly of 74% of newly diagnosed patients for T1D and 26% of confirmed diagnostic patients, of whom 52% are females. The mean age of diagnosis is 7 ± 4 years, the mean of HbA1c at the time of diagnosis is 11.63 ± 2.16%, and the percentage of family history in our series is 69%. The proportion of positive results for Anti-IA2 antibodies and Anti-GAD antibodies are respectively 76.92% and 62.82%, and 52.56% of patients are positive for both autoantibodies. **Conclusion :** This study confirmed the diagnosis and the classification of T1D (type 1A) in 87.18% of patients, and we reported that the prevalence of Anti-GAD and Anti-IA2 is higher in girls than in boys.

P.A3.03.03

Analysis of B cell lymphocyte subpopulations in pre and post dialysis end stage renal disease patients

D. Daikidou[1], *A. Fylaktou*[2], *M. Stangou*[1], *D. Asouchidou*[2], *V. Nikolaidou*[2], *E. Sampani*[1], *F. Markovasili*[2], *T. Chronis*[2], *C. Dimitriadis*[1], *A. Papagianni*[1];
[1]*Department of Nephrology, Aristotle University of Thessaloniki, Thessaloniki, Greece,* [2]*Department of Immunology, National Peripheral Histocompatibility Center, Hippokration Hospital, Thessaloniki, Greece.*

Introduction and aims: End-stage renal disease (ESRD) is associated with immunodeficiency, which makes a significant contribution to morbidity and mortality. The present study aimed at analysis of B lymphocyte subpopulations in pre- and six months post-dialysis ESRD patients. **Methods:** B cells (CD45+ CD19+) and their subsets B1a (CD19+CD5+), naive (CD19+ CD27−), memory (CD19+ CD27+), CD19+ BAFF+ and CD19+ IgM+, were quantified using flow cytometry in the peripheral blood of 27pre-dialysis and 11 post-dialysis patients.The results were compared to healthy control group. **Results:** ESRD patients had reduced lymphocyte count (1606±655μ/L vs.2459±520μ/L, p<0.001)and B cell (CD19+) count (82.7±54.9μ/L vs.177.6±73.8μ/L, p<0.001)compared to controls. Likewise, whereas the percentages of B cell subsets were not particularly affected, except for B1a subset which presented a significant increase (4.1±3.8% vs. 0.7±0.7% p<0.001), the absolute number of almost all subsets was significantly smaller in ESRD patients (CD19+: 81.3±60.4μ/Lvs. 162.1 ±64.5μ/L, p=0.005, Naive:55.6±46.6μ/Lvs.97.2±46.6μ/L, p=0.004, Memory: 27.1±15.6μ/L vs.83.5±56.8μ/L, p<0.001,CD19+BAFF+:69.5±47.5μ/Lvs. 154.7±74.6μ/L, p<0.001, CD19+IgM+: 58.1±42.7μ/Lvs.117.9±58.94μ/L, p=0.001). In 11 patients who had a follow-up 6 months after starting on renal replacement treatment no differences were found, apart from CD19+IgM+ (74.7±7.4μ/Lvs.post 66.9±14.7μ/L, p=0.041) and B1a percentage (3.0±2.4% vs. 1.0±0.8% p=0.038), which further decreased. **Conclusions:** Significant reduction was noticed in B cells subpopulations in patients with ESRD on pre-dialysis stage, and in some of them further reduction was noticed in post-dialysis stage, and these changes may be implicated in clinical manifestations, such as frequent infections or impaired response to vaccination.

P.A3.03.04

The introduction of*N*-linked glycans in the variable domain of Anti-Citrullinated Protein Antibodies marks the development of Rheumatoid Arthritis.

L. Hafkenscheid[1], *E. de Moel*[1], *I. Smolik*[2], *B. Jansen*[3], *A. Bondt*[1], *M. Wuhrer*[1], *T. Huizinga*[1], *R. Toes*[1], *H. Scherer*[1], *H. El-Gabalawy*[2];
[1]*LUMC, Leiden, Netherlands,* [2]*University of Manitoba, Winnipeg, Manitoba, Canada,* [3]*Ludger Ltd., Culham Science Centre, Oxfordshire, United Kingdom.*

The most prominent biomarker implicated in disease pathogenesis in Rheumatoid arthritis (RA) are anti-citrullinated protein autoantibodies (ACPA). Intriguingly, ACPA can be present years before the disease onset. Therefore it is thought that the development of ACPA-positive disease is a multistep process. The possible sequence of events starts with an environmental event (first hit) that initiates a break of tolerance against citrullinated antigens leading to ACPA production. Upon a certain trigger (second hit) ACPA-B cells receive T-cell help inducing an autoimmune responses resulting in RA. We recently showed that ACPA-IgG display a unique feature by harbouring *N*-linked glycans in the antigen-binding fragment (Fab) and the *N*-glycosylation sites were not germ-line encoded but introduced upon SHM, indicating T-cell help. Therefore we aimed to investigate if the presence of ACPA-Fab-glycosylation is an indicator of the second hit. We analysed the ACPA-IgG Fab-glycosylation in indigenous North-American first-degree relatives (FDR) that developed RA overtime and in ACPA-positive FDR that did not. The data showed that ACPA-positive individuals that stayed healthy had indeed lower levels of ACPA-Fab-glycosylation compared to ACPA-positive FDR that developed RA. This indicates that glycans are involved in the disease progression and could predict when an ACPA-positive individual is at risk to develop disease.

P.A3.03.05

Clinical usefulness of autoantibodies to M-type phospholipase A2 receptor for diagnostic and monitoring disease activity in idiopathic membranous nephropathy

***d. khelifi touhami**, y. lounici, s. Amoura, M. Benhalima;*
Mustapha bacha teaching hospital, Algiers, Algeria.

Objectives PLA2R is the major target antigen in adult idiopathic membranous nephropathy (iMN). This study aimed to assess these antibodies (Abs) prevalence and specificity in a cohort of iMN Algerian patients and to correlate this Abs with clinical parameters reflecting the disease activity. **Patients and methods** We measured anti-PLA2R Abs using an immuno-enzymatic assay in the serum of 40 patients with iMN, 09 with secondary MN and 10 with other forms of primary glomerular diseases.
Anti-PLA2R Abs levels were correlated with proteinuria, serum albumin and serum creatinine in IMN patients. In 6 anti-PLA2R positive iMN patients, Abs levels were assessed at various stages of clinical disease and correlated with disease activity. **Results** Anti-PLA2R Abs were detected in 57,5% of iMN patients, but not in secondary MN or other forms of primary glomerular diseases. In 24 iMN patienst, proteinuria was >3g/24h at the time Abs measurement. 23 (91,66%) of them were positive for PLA2R Abs, while in 14 patients, proteinuria was < 3g/24h. Among them, only 1 patient was positive for Abs. In iMN patients, Abs levels correlated positively with proteinuria and negatively with serum albumin. No correlation was found between Abs and serum creatinine. During the clinical course of the 6 anti-PLA2R positive patients, Abs levels correlated with clinical status, which were high at the active phase disease and decreased during remission. **Conclusions** anti-PLA2R Abs is a sensitive and specific test for iMN. Abs levels correlate with clinical disease activity, there measurement may provide a tool for monitoring disease activity.

P.A3.03.06

Gene expression profile of tolerogenic dendritic cells differentiated with vitamin D3, dexamethasone and rapamycin

***J. Navarro-Barriuso**[1], M. Mansilla[1], B. Quirant-Sánchez[1], A. Teniente-Serra[1], Á. Sánchez-Pla[2], M. Naranjo-Gómez[1], C. Ramo-Tello[3], E. Martínez-Cáceres[1];*
[1]Germans Trias i Pujol University Hospital and Research Institute, Campus Can Ruti, Badalona, Spain, [2]Department of Statistics. University of Barcelona, Barcelona, Spain, [3]Germans Trias i Pujol University Hospital, Campus Can Ruti, Badalona, Spain.

Background: Tolerogenic dendritic cell (tolDC)-based therapies have become promising approaches for the treatment of autoimmune diseases by their potential ability to restore immune tolerance in an antigen-specific manner. There is a broad variety of protocols to generate tolDC *in vitro*, being their differentiation in the presence of vitamin D3 (vitD3-tolDC), dexamethasone (dexa-tolDC) or rapamycin (rapa-tolDC) three of the most frequent. However, the characteristics of these cells are very heterogeneous, thus making the need to find common genetic pathways and biomarkers of high relevance.
Objective: To compare the transcriptomic profile of vitD3-tolDC, dexa-tolDC and rapa-tolDC in order to find common induced pathways and biomarkers.
Methods: Monocyte-derived dendritic cell differentiations of immature (iDC), mature (mDC), vitD3-tolDC, dexa-tolDC and rapa-tolDC from 5 healthy donors were generated, and a microarray analysis was performed (Affymetrix). Results were normalized and filtered, and differentially expressed genes (DEG) were selected. A Gene Set Enrichment Analysis (GSEA) was performed to select common enriched pathways. Statistical analyses were performed using R software.
Results: Common DEG could not be found for the three tolDC, although 14 genes (many of them immune-related) appeared up-regulated in at least one condition. GSEA revealed 11 common protein sets differentially expressed in tolDC. However, all of them were induced for vitD3-tolDC and dexa-tolDC, while down-regulated in rapa-tolDC.
Conclusions: The analysis revealed that, despite not sharing potential common biomarkers, vitD3-tolDC and dexa-tolDC presented similar transcriptomic profiles, suggesting an induction of immune tolerance through common pathways, while rapa-tolDC seem to develop their function through different ones.

P.A3.03.07

Higher responsiveness of CLL cells to B-cell receptor stimulation is associated with reduced expression of inhibitory molecules of the NF-κB pathway

***R. W. J. Meijers**[1], A. F. Muggen[1], L. G. Leon[1], O. B. Corneth[2], R. W. Hendriks[2], J. J. van Dongen[1], A. W. Langerak[1];*
[1]Dept. of Immunology, Erasmus MC, Rotterdam, Netherlands, [2]Dept. of Pulmonary Medicine, Erasmus MC, Rotterdam, Netherlands.

Background Chronic lymphocytic leukemia (CLL) is a heterogeneous disease based on both clinical and biological characteristics. We previously described (Muggen et al, Leukemia, 2015) differences in Ca^{2+}-levels among CLL cases (both basal and upon B-cell receptor (BCR) stimulation). Such differences in BCR responsiveness could reflect a heterogeneity in CLL pathogenesis due to cell-intrinsic factors.
Aim To elucidate cell-intrinsic differences between BCR-unresponsive and BCR-responsive CLL patients.
Methods From 52 CLL cases, the BCR-responsiveness was determined *ex vivo* based on Ca^{2+}-influx upon α-IgM stimulation. Phosphorylation levels of various BCR-signaling molecules, as well as the expression of activation markers were assessed by flow cytometry. Transcription profiling of BCR-responsive (n=6) and BCR-unresponsive CLL cases (n=6) was performed by RNA sequencing. RQ-PCR was used to validate transcript level differences.
Results The increase in Ca^{2+} after α-IgM stimulation was accompanied by higher phosphorylation of PLCγ2, Akt and higher surface expression levels of CD21, CD38, CD80 and CD27. RNA sequencing revealed differences between the two groups, especially in expression of NF-κB pathway genes. RQ-PCR validation in additional CLL cases confirmed the lower expression of the critical NF-κB inhibitors *NFKBIB* (p=0.021) and *NFKBIE* (p=0.009) genes in BCR-responsive CLL. Likewise, expression of the potential NF-κB inhibitors *NFKB1* (p=0.017) and *NFKB2* (p=0.009) was reduced.
Conclusion From our data we conclude that BCR-responsive CLL cells have a more activated cell surface phenotype and reduced expression of components that are associated with inhibition of NF-κB signaling. These findings illustrate that enhanced NF-κB activation is critical for the BCR responsiveness of CLL cells.

P.A3.03.08

Circulating Dendritic cells and monocyte subsets in multiple sclerosis patients

***A. Monteiro**[1,2], C. Cruto[3], P. Rosado[3], L. Rosado[3], M. Fonseca[1], A. Paiva[4];*
[1]CICS-UBI- Centro de Investigação em Ciências da Saúde, Covilhã, Portugal, [2]Serviço Patologia Clínica, Centro Hospitalar Cova da Beira, Covilhã, Portugal, [3]Serviço Neurologia, Centro Hospitalar Cova da Beira, Covilhã, Portugal, [4]Unidade de Gestão Operacional de Citometria, Serviço de Patologia Clínica, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal.

Dendritic cells (DCs) and monocyte have been considered key players in multiple sclerosis (MS), an autoimmune demyelinating disease of the central nervous system. We propose to characterize phenotypically circulating DCs and monocytes subsets in remission and relapsing RR (relapsing-remitting)MS patients treated with interferon beta (IFN-β) comparing with healthy controls. 38 patients with RRMS, 30 of them in remission and 8 in relapse were included in this study, as well as, 20 healthy age- and gender-matched volunteers. The study was approved by Centro Hospitalar Cova da Beira Ethics Committee. By flow cytometry, and based on the expression of CD123, HLA-DR, CD14, CD16 and CD33, were identified and characterized: plasmacytoid DC (pDC), myeloid DC (mDC), classical monocytes, intermediate monocytes (HLA-DR^{dim+} or HLA-$DR^{bright+}$) and non-classical monocytes. Moreover, the expression of CD11c, CD54 and CD123 was evaluated in all subsets. The ratio mDC/pDC was significantly decreased in remission when compared to relapsing patients and healthy controls. Concerning monocytes subpopulations, we observed a decrease in the frequency and absolute number of non-classical monocytes in both disease stages. Interestingly, a bimodal expression of CD54 on pDCs, decreasing in remission and increasing in relapse RRMS, was observed. In the two groups of patients, intermediate monocytes HLA-$DR^{bright+}$ exhibit an increased expression of CD11c, and in relapsing phase a decreased expression of CD123. Alterations in the homeostasis of circulating DCs and monocyte subsets in RRMS patients supports the idea that these cells participate in the pathophysiology of MS and, at least in part, were regulated by the IFN-β treatment.

P.A3.03.09

Chronic Lymphocytic Leukaemia: Increased IL-10 and STAT3 Levels in B cells

***Ö. ÖZCAN**[1], M. GELMEZ[1], S. CİNAR[1], G. DENIZ[1], M. AKTAN[2];*
[1]Istanbul University Aziz Sancar Institute of Experimental Medicine, İstanbul, Turkey, [2]Istanbul University, Istanbul Medical Faculty, Department of Internal Medicine, İstanbul, Turkey.

Chronic Lymphocytic Leukaemia **(**CLL) is characterized by the accumulation of $CD5^+CD19^+$ B cells in the bone marrow and peripheral blood. Recent studies indicated that expression of IL-10, AID and mir-155 that are regulated by STAT3 are increased in CLL patients. $CD5^+CD19^+$ regulator B (B_{reg}) cells secrete IL-10 and suppress the immune system. While the CLL cells show similar immunophenotypic properties to Breg cells, they are also thought to be functionally similar. In this study, levels of STAT3 and IL-10 in CLL patients were investigated. Peripheral blood samples obtained from patients (n: 24) and healthy subjects (n: 14). Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll gradient method. PBMCs ($1x10^6$ cell/ml) were cultured for 48 hours in the presence and absence of CpG (1 ug/ml) for IL-10 expression and for STAT3 expression cultured with and without PMA (1 ug/ml) for 15 min. IL-10 and STAT3 expressions were analysed using anti-CD5, anti-CD19, anti-CD38, anti-STAT3 and anti-IL-10 monoclonal antibodies by flow cytometry. Compared to healthy subjects, in lymphocyte population increased $IL\text{-}10^+$, $IL\text{-}10^+CD19^+$ and $STAT3^+CD19^+$ cells were obtained ($p<0.0001$, $p<0.0001$ and $p<0.0001$, respectively). $CD19^+$ B cells showed elevated IL-10 content in CLL patients ($p<0.0001$). Similarly, increased IL-10 levels ($p<0.0001$) were also found in $CD5^+CD19^+$ cells, whereas STAT3 levels were diminished ($p<0.0074$). These results support that for the levels of IL-10 and STAT3 in CLL patients, B cells are clearly different from normal B-lymphocytes might have a role in the biology of CLL.

P.A3.03.10

The role of Natural Killer cells in B cell Acute Lymphoblastic Leukemia

***G. Ozcit**, G. Deniz, Adın-Cınar Suzan, Gelmez M. Yusuf, Yılmaz Abdullah, Aydoğan Gonul;*
Aziz Sancar Institute of Experimental Medicine, Istanbul, Turkey.

In ALL lymphocyte progenitor cells' maturation process break down and proliferation become uncontrollable. Studies suggesting the use of NK cells capable of lysing tumor cells in the treatment of ALL. In this study, the response of NK cells to cytokines in B-ALL cases and the correlation with assessment of minimal residual disease (MRD) by flow cytometry according to International BFM Study Group protocol were investigated. CD7, CD10, CD19, CD34, CD45 cell surface expressions were evaluated to detect B-ALL blast phenotypes. CD3, CD16 and CD56 cell surface expressions were detected in BM and peripheral blood (PB) samples of B-ALL. Mononuclear cells, isolated from PB and BM obtained from B-ALL patients at day of diagnosis, were stimulated with IL-2 for 24 hours and PMA+IO for 5 hours. Intracellular IFN-γ and IL-10 levels in NK cell subsets were determined by flow cytometry. PB $CD3^-CD56^{dim}CD16^-$ and $CD3^-CD56^{bright}CD16^+$ NK cell frequencies were found to be higher than those from BM. Although IL-10 content of $CD3^-CD56^{dim}CD16^+$ and $CD3^-CD56^{bright}CD16^+$ NK cell subsets were decreased, IFN-γ content of both subsets of NK cells were diminished after stimulation both with IL-2 and PMA-IO. After treatment on day 15, IFN-γ levels of $CD3^-CD56^{dim}CD16^+$ NK cells were elevated by IL-2 stimulation only in patients detected as low risk. . Our findings support that increased IFN-γ secreting $CD3^-CD56^{dim}CD16^+$ NK cell subset in FLR patients, after treatment of B-ALL according to the International BFM Study Group protocol, might have an indicator for the following of MRD-ALL at FLR patient.

P.A3.03.11

The Role of Phagocytic Cells in Acute Respiratory Distress Syndrome

***A. Petre**[1], A. Ohradanova-Repic[1], K. Krenn[2], R. Ullrich[2], K. Markstaller[2], H. Stockinger[1];*
[1]Center for Pathophysiology, Infectiology and Immunology, MUW, Vienna, Austria, [2]Department of Anesthesia, General Intensive Care and Pain Medicine, MUW, Vienna, Austria.

Acute respiratory distress syndrome (ARDS) remains a disputed clinical entity in terms of definition, pathophysiological understanding and therapeutic approach. Due to high heterogeneity, ARDS subgroups are best studied individually. Our focus lies on a) ARDS associated with pneumonia and b) ARDS in trauma patients. By means of multicolor flow cytometry, we provide a comprehensive analysis of surface markers in sputum, tracheal aspirate and bronchoalveolar lavage (BAL) cells. Sputum is obtained from lung-healthy volunteers, whereas tracheal aspirate and BAL are collected from lung-heathy intubated patients, as well as from patients diagnosed with ARDS. By comparing samples obtained from lung-healthy controls to those of ARDS patients, we aim to pinpoint cell populations that undergo significant changes in pathological states, with a focus on phagocytic cells (especially granulocytes and macrophages). Once we identify the populations most relevant for the studied condition, we proceed to performing mRNA-Sequencing of the fluorescence-activated cell-sorted phagocytes. By characterizing the expression patterns in ARDS patients depending on the underlying pathology and by discriminating these changes from those induced by mechanical ventilation, this study will provide a basis for identifying individual risk factors and biomarkers of lung disease on the intensive care unit.

P.A3.03.12

Determination of quantity and biological activity of platelet-bound antibodies using Fc-receptors in a single assay using surface plasmon resonance

***Z. Szittner**[1], R. Temming[1], D. Schmidt[1], A. Bentlage[1], R. Visser[1], S. Lissenberg-Thunnissen[1], J. Mok[1], W. J. van Esch[1], M. Sonneveld[1], E. de Graaf[1], M. Wuhrer[2], L. Porcelijn[1], M. de Haas[1], E. van der Schoot[1], G. Vidarsson[1];*
[1]Sanquin, Amsterdam, Netherlands, [2]Leiden University Medical Center, Leiden, Netherlands.

Here we describe how surface plasmon resonance biosensor array can be utilized to characterize and determine the biological activity of platelet-bound antibodies. Current methods applying anti-human IgG using whole platelets measure both antibodies targeting the platelets and those bound to platelet FcγRIIa. We hypothesized that this can be overcome using a human FcγR for detection of only Fab-bound antibodies targeting platelets. At the same time this can provide an evaluation reflecting the biological activity of the platelet-bound antibodies. This would improve the diagnostic work up both in fetal and neonatal alloimmune thrombocytopenia (FNAIT) and in immune thrombocytopenia (ITP). To test our hypothesis we compared the binding of chloroquine-treated platelets (removing HLA antigens), opsonized with serum samples from 166 women with anti-HPA-1a antibodies causing FNAIT, to anti-IgG and to FcγR coupled onto the biosensor surface. Our results show that compared to anti-IgG, the binding by FcγR of these opsonized platelets shows a stronger correlation (Pearson's r=0.56 and 0.67, respectively) to the anti-HPA-1a antibody levels measured by monoclonal-antibody immobilization of platelet antigens (MAIPA). In addition, we have combined this technique with anti-complement (C3) and anti-IgA and anti-IgM and show that these features can also be evaluated simultaneously and directly on primary material from patients with ITP (n> 50). These methods show that SPR technology can be applied to get reliable clinically relevant data that go beyond what is possible with current diagnostic techniques.

P.A3.03.13

Heavy/light chain (HLC) ratio measurements in intact immunoglobulin multiple myeloma (IIMM) patients-a single center experience

*I. Kakkas[1], I. Konstantellos[2], S. Delimbasi[2], K. Papageorgiou[1], N. Harhalakis[2], **A. Tsirogianni**[1];*
[1]Immunology-Histocompatibility Dept. "Evaggelismos" General Hospital, Athens, Greece, [2]Hematology & Lymphoma Dept. "Evaggelismos" General Hospital, Athens, Greece.

Aim: The investigation for existence of any prognostic significance of HLC measurements for symptomatic IIMM patients (before treatment initiation) diagnosed and treated in our Hospital.
Patients-Methods: Forty-one newly diagnosed symptomatic IIMM patients were studied. Twenty-five of them were men and 16 women. Their median age was 68 years (range: 43-83). The isotype of paraprotein was in 31 cases IgG and in 10 cases IgA. Patients median follow-up was 16 months (range: 6-24). HLC ratio was determined in all patients before treatment initiation. HLC measurements were performed by using the Hevylite™ assays (The Binding Site Group Ltd, UK) on a SPA PLUS turbidometer.
Results: Statistical analysis was done by using the x^2 test. At the time of last evaluation, 36 patients were alive. Five patients had died due to disease progression and their median survival was seven months (range: 2-14). Extreme HLC ratios (<0.01 or >200) emerged in 14 patients (7/31 IgG and 7/10 IgA, $p < 0.05$). Two out of five deceased patients were IgG and three IgA. Also, four out of the five deceased patients had extreme HLC ratios ($p < 0.05$). It is noted that all three IgA deceased patients emerged extreme HLC ratios ($p < 0.01$).
Conclusions: Despite the limited number of patients in our study, it is clear from the above-mentioned that there is a statistically significant correlation between IgA isotype of paraprotein and HLC ratio extreme values (<0.01 or >200). Also, there is a statistically significant correlation between mortality and HLC ratio extreme values, especially for IgA patients.

P.A3.03.14

Study of soluble vascular endothelial cell adhesion molecules sICAM-1 and sVCAM-1 in hemodialysis patients

*C. Tsigalou[1], T. Konstantinidis[1], **A. Tsirogianni**[2], G. Romanidou[3], K. Kantartzi[3], A. Grapsa[1], M. Panopoulou[1], P. Pasadakis[3];*
[1]Laboratory of Microbiology-Democritus University of Thrace, ALEXANDROUPOLIS, Greece, [2]Immumology-Histocompatibility Dept. Evangelismos General Hospital, Athens, Greece, ATHENS, Greece, [3]Division of Nephrology, Democritus University of Thrace, Alexandroupolis, Greece, ALEXANDROUPOLIS, Greece.

Aim: Cardiovascular complications are the leading cause of mortality and morbidity in patients with end-stage renal disease undergoing chronic hemodialysis (ESRD-HD). Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are involved as markers of the atherosclerotic burden.The aim of the study was to determine the level of sICAM 1 and sVCAM-1 in patients with ESRD-HD.
Materials and Methods: The study enrolled 60 patients, mean age 64±13 yrs, ♀ 39(65%) and ♂ 21(35%). The levels of sICAM-1and sVCAM-1 were measured at the beginning and after 6 months by elisa kit (Bender MedSystem, Austria).
Statistical analysis was performed with OriginPro version 8. Student t-test and one way ANOVA were used for data that followed Gaussian distribution, while the Kruskal-Wallis tests were used for data that did not follow the Gaussian distribution. For all measurements, a two-tailed p value ≤0.05 was considered as significant.
Results: The mean value of sICAM-1and sVCAM-1at the start of dialysis was 442.01±215.5ng/mland 11704,83 ±421,4ng/ml respectively. The levels of sICAM-1 after 6 months increased significantly (442.01±215.5ng/ml vs 835,08 ±339ng/ml p<0.0001), but not the levels of sVCAM-1(11704,83 ±421,4ng/ml vs 10461,31±314.7ng/ml p=0,072). Moreover,sICAM-1 was elevated significantly in dialysis patients with co-morbidity of coronary artery disease and diabetes mellitus type II (442.01±215.5ng/ml vs 631,28±134ng/ml =0,089).
Conclusions: A significant increase sICAM-1 at 6 months compared with baseline was observed as evidence for chronic inflammatory/immune system involvement. The role of sVCAM-1 and sICAM-1 as markers of atherosclerosis in chronic renal disease may implicate an upregulation of inflammatory/immune response and may facilitate possible therapeutic interventions.

P.A3.03.15
Incidence of CMV infection in childhood

N. Zotos[1], *P. Christodoulou*[1], *E. Tatsina*[2], *N. Varsamis*[1], *A. Zotou*[3], *F. Adam*[1], *G. Katagis*[1], *L. Papageorgiou*[1], *M. Gianniki*[4], *N. Tsifetaki*[1];
[1]*General Hospital of Ioannina, Greece, Ioannina, Greece,* [2]*Papageorgiou Hospital, Thessaloniki, Greece,* [3]*University Hospital of Ioannina, Ioannina, Greece,* [4]*Agia Sofia Hospital, Athens, Greece.*

Aim: To determine the incidence of CMV infection in children during a two-year period (2016-2017) Method: 980 children of 1-14 years of age who presented to the outpatient department of the hospital with symptoms of the disease or hospitalized from January 2016 until December 2017, were the material of the study. The samples were tested for IgG and IgM antibodies against CMV by ELISA (AxSYM,Abbott).The diagnosis was set either by detecting both IgG and IgM antibodies or by detecting a fourfold elevation of the titer of IgG antibodies in the second serum sample Results: No antibodies were detected in 520 (53%) out of 980 children that were tested for CMV antibodies, while 460 (47%) were positive. 232 (50.4%) were suffering from CMV infection.
139 (59.9%) out of 232 were initially positive only for IgM antibodies, 73 (31.5%) were positive for IgG and IgM antibodies while 20 (8.6%) were positive only for IgG antibodies and presented a fourfold increase of their titer in the second sample. The remaining 228 patients (49.6%) presented a low titer of IgG antibodies in both serum samples. There was no significant difference in two sexes. Epidemiological factors, such as social-economic status, follow-up in daily care centers and cohabitation in large numbers don't correlate with CMV serum-positivity. Conclusion: The study revealed that CMV infection is not rare. Due two severe complications in infancy, it is important to diagnose the disease quickly and efficiently so that proper personalized care is delivered.

P.A3.03.16
Estimation of two serological markers in the diagnosis of rheumatoid arthritis

N. Zotos[1], *P. Christodoulou*[1], *E. Tatsina*[2], *N. Varsamis*[1], *A. Zotou*[3], *G. Katagis*[1], *F. Adam*[1], *A. Pournou*[1], *M. Gianniki*[4], *N. Tsifetaki*[1];
[1]*General Hospital of Ioannina, Greece, Ioannina, Greece,* [2]*Papageorgiou Hospital, Thessaloniki, Greece,* [3]*University Hospital of Ioannina, Greece, Ioannina, Greece,* [4]*Agia Sofia Hospital, Athens, Greece.*

Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology Aim: To determine the frequency of antibodies against synthetic circular citrullinyl peptides (anti-CCP) and to compare them with rheumatoid factor (RF) in a group of patients with rheumatoid arthritis symptoms. Material-Method: 74 sera that were referred to the Laboratory with symptoms of mild to very severe rheumatoid arthritis were tested for the presence of anti-CCP and IgM RF antibodies. Control of the first was performed with enzyme immunoassay anti-RA / CCP τ and of the second with the method of nebulometry. Results: In 44 out of 74 patients the titles of both antibodies ranged in normal levels. Of the others, 18 had elevated titles of both antibodies while IgM were detected in 12. The 44 patients with negative antibody titles and 12 with only RF antibodies positive, showed mild and non-specific symptoms of the disease whereas the 18 patients with elevated anti-CCP and IgM RF titles had clinical picture consistant with rheumatoid arthritis. Conclusions: Anti-CCP antibodies are an important and specific indicator in the diagnosis of rheumatoid arthritis. Rheumatoid factor, although a sensitive marker for RA, lags behind in its specificity because it is detected in small percentages in a healthy population and other autoimmune diseases.

P.A3.03.17
Incidence of antibodies against Toxoplasma gondii,Rubella virus,cytomegalovirus and herpes simplex virus in greek and foreign women

N. Zotos[1], *E. Tatsina*[2], *L. Papageorgiou*[1], *E. Chrysostomou*[1], *P. Christodoulou*[1], *A. Zotou*[3], *N. Varsamis*[1], *A. Pournou*[1];
[1]*General Hospital of Ioannina, Greece, Ioannina, Greece,* [2]*Papageorgiou Hospital, Thessaloniki, Greece,* [3]*University Hospital of Ioannina, Ioannina, Greece.*

Aim: To determine the incidence of infections caused by Toxoplasma.gondii, Rubella Virus, Cytomegalovirus (CMV) and Herpes Virus Simplex (HSV) in Greek and foreign women, presenting in a tertiary hospital of North-Western Greece. Material and Methods: 382 women (278 Greeks and 104 Foreigners leaving in Greece) were tested for antibodies against T.gondii ,Rubella Virus,CMV, HSV during one year (January 2017 to December 2017).327 were tested for antibodies against T.gondii, 238 were tested for antibodies against Rubella Virus,302 were tested for anti-CMV antibodies and 124 for anti-HSV antibodies.92 of the women were tested for antibodies against all of the pathogens (TORCH). An enzyme-linked immunoassay was employed for the detection of all antibodies. Results: It is important to note that there was a statistically significant difference in the seropositivity against anti-Toxoplasma IgG and anti-CMV IgG antibodies in foreign women in comparison to Greek women (19.8% and 10.2%, 88.6% and 71.8%,) .There was no difference as far as positivity to the remaining types of antibodies tested in foreign and Greek women is concerned. Conclusion: An elevated seropositivity against T.gondii, Rubella Virus, CMV and HSV detected in women, both Greek and Foreign, makes the evaluation of the population inevitable. In this way infection prevention and control can be accomplished.

P.A3.03.18
Rapid Target Identification for T-Cell Immune Responses with SpotMix™ Peptide Pools

U. Reimer, *M. Eckey, P. Holenya, T. A. Teck, J. Zerweck, T. Knaute, H. Wenschuh, T. Kaan, F. Kern;*
JPT Peptide Technologies, Berlin, Germany.

Introduction: Vaccinations inducing or boosting T-cell immunity are successfully used in the infectious diseases and cancer fields. Suitable vaccine target proteins must contain T-cell stimulating peptides providing good epitopes. However, to date, algorithms for T-cell epitope prediction cannot reliably identify the most promising target proteins. Protein-spanning, overlapping pools of conventional synthetic peptides (PepMix™) represent all possible stimulating peptides in a target protein and are, therefore, ideally suited for this purpose. However, they are too costly for testing multiple potential target proteins.
Methods: Based on a method for the highly parallel synthesis of multiple peptides in low quantities, referred to as SPOT synthesis, we developed a novel protocol for synthesizing multiple peptides, which includes QC and quantification by LC-MS and permits the production of equimolar pools of SPOT peptides (SpotMix™). Our new protocol features consistent quality control of thousands of peptides.
Results: Comparisons between T-cell stimulation assays (ELISPOT, flow cytometry/intracellular cytokine staining) performed with the classic PepMix™ peptide pools and SpotMix™ pools demonstrated similar performance of these preparations.
Conclusions: SpotMix reagents will significantly facilitate T-cell protein target discovery by permitting the synthesis of protein-spanning, overlapping peptide pools for many potential target antigens in parallel. These can be tested experimentally and the most promising target proteins can be selected for further investigation. Since SPOT peptides are unpurified and produced in small quantities (approx. 10nmol) they are unsuitable for T-cell expansions or other therapeutic applications, however, represent ideal tools for T-cell target discovery.

P.A3.03.19
Fine characterization of healthy Conjunctiva: Main differences when comparing IELs and peripheral blood lymphocyte subsets.

Z. José Carlos, *C. Alfredo;*
Universidad de Valladolid, Valladolid, Spain.

Introduction: As occurs in other mucosal tissues —for example gut, bronchi and nose—, ocular mucosa holds a conjunctiva- associated lymphoid tissue (CALT). It is well-known that MALT (Mucosa Associated Lymphoid Tissue) has morphological and functional variations across tissues. Therefore, a thorough analysis of lymphoid populations might render useful information on ocular surface conditions. **Objectives:** The aim of this study is to improve the knowledge of human immune system within the conjunctiva in different ocular surface conditions. **Material and methods:** Twenty-five healthy volunteers were recruited. Peripheral blood lymphocytes were obtained by venipuncture while intraepithelial lymphocytes (IELs) from conjunctival mucosa were obtained by brush cytology. Major and fine subsets were characterized by flow cytometry. Memory, naïve, γδ T cells, $CD8^+$ (**Tc, NKT subtypes**), $CD4^+$ (**Th0, Th1, Th2, Th17, Th1/Th17, Th22** and **Treg** subsets), B cells (B_1 and B_2) and **NK cells** —regulatory and cytotoxic— subsets were analyzed in both conjunctival mucosa and peripheral blood. **Results:** Age and sex seemed to determine few differences in some lymphocyte subsets: Th1 cells might be age-influenced whereas Th22 might be sex-influenced. As expected, no strong correlations between peripheral and conjunctival lymphocytes were found. Conjunctival T cells seemed to be mainly CD8+ and TCRγδ+, while they were only a minor population in peripheral blood. Memory CD4+ T cells, NKT, B1, Tregs and regulatory NK cells had higher values in conjunctiva. **Conclusions:** Some well-known differences (increased TCRγδ cells) whereas others are apparently new in our knowledge (increased B1, NKT, Tregs and regulatory NK lymphocytes) were found. These features provide an extra effect and regulatory function to the conjunctiva.

P.A3.03.20

Cell-free tumoral DNA (ctDNA) based *EGFR* mutation analysis in a cohort of Indian non-small cell lung carcinoma (NSCLC) patients.

***S. Verma Kumar**, R. Katara, V. Kumar, J. Kandpal, S. Pandey, S. Sharma, L. Kini;*
CORE Diagnostics, Gurugram, Haryana 122016, India., Gurugram, India.

Patients with lung cancer show poor prognosis, with a five-year survival rate of about 17.8%. Epidermal Growth Factor Receptor (*EGFR*) mutation analysis is critical for patient selection for targeted tyrosine kinase inhibitor (TKI) therapy, and immunotherapy. Mutations such as exon 19 deletions and L858R point mutation confer sensitivity to EGFR TKIs; while mutations such as T790M confer decreased sensitivity to first- and second-generation *EGFR* TKIs.
The study population comprised of 627 (male: female ratio of 1.18: 1) patients with primary NSCLC. The patients ranged in clinical presentation from Stages I to IV of lung cancer, and most had previously tested positive in *EGFR* mutation analysis on tissue biopsy. The ctDNA was tested for "hotspot mutations" in *EGFR* c.2573T>G (L858R); *EGFR* Deletion E746-A750; and *EGFR* c.2369C>T (T790M) using ddPCR.
We report a 45.15% positivity for *EGFR* mutations in this cohort of NSCLC patients. Further, 26.63% tested positive for Deletion E746-A750; 21.37% for T790M; and 13.87% for L858R mutations. 11.16% cases showed concomitant positivity for Deletion E746-A750 and T790M, and 4.62% for L858R and T790M. As the sensitivity of ctDNA based *EGFR* testing ranged from 71-74% (as against tissue-based mutation analysis), we recommend testing all negative cases by a repeat tissue biopsy. We are currently carrying out a retrospective correlation of these test results with patients' clinical data, especially with reference to disease progression.

P.A3.03.21

Distinct cytokine patterns may regulate the severity of perinatal asphyxia

***A. Bajnok**, L. Berta, C. Orban, G. Toldi;*
Semmelweis University, Budapest, Hungary.

Neuroinflammation following perinatal asphyxia may have dual aspects being a hindrance, but also a necessity in the recovery of the CNS. We aimed to assess intracellular cytokine levels of T-lymphocytes and plasma cytokine levels in moderate and severe asphyxia in order to identify factors that may influence patient outcome. We analyzed data of neonates with moderate (n = 17) and a severe (n = 11) asphyxia. Grouping was based on neuroradiological and aEEG characteristics. Blood samples were collected at 6 h, at 24 h, 72 h, 1 week and 1 month of life. Blood samples were stimulated for 6 h, then intracellular cytokine levels were determined using flow cytometry. Cytokine plasma levels were measured using Bioplex immunoassays. The prevalence of IL-1b+ CD4 cells was higher in severe than in moderate asphyxia at 6 h and the prevalence of CD4+ IL-1β+ and CD4+ IL-1β+ CD49d+ cells appears to be able to predict severity of the insult at an early stage in asphyxia. At 1 mo, intracellular levels of TNF-α were higher in the severe group. Plasma IL-6 levels were higher at 1 wk in the severe group and decreased by 1 mo in the moderate group. Intracellular TGF-β levels were increased from 24 h onwards in the moderate group only. IL-1β and IL-6 appear to play a key role in the early events of the inflammatory response, while TNF-α seems to be responsible for prolonged neuroinflammation, potentially contributing to a worse outcome. TGF-β has a compensatory role in decreasing inflammation.

P.A3.03.22

Immunobiogram as a diagnostic assay for detection of resistance to immunomodulatory treatment in patients with chronic inflammatory diseases.

***Á. Ortega**, M. Di Scala, T. Yebra, T. Diez, J. Richter, I. Portero;*
BIOHOPE Scientific SL, Madrid, Spain.

Introduction: The immune system is very complex, dynamic and with self-learning capacity. The response of patients with chronic inflammatory diseases treated with immunomodulatory drugs can change over time.
Resistance arise during immunomodulator treatment and is particularly problematic during long-term treatment regimens. Nowadays, there are no biomarkers or pharmacovigilance tools to monitor drug resistance development.
Methods: Biohope has designed the Immunobiogram to be a "final point" immunoassay using a pharmacodynamic approach. We studied a cohort of transplant recipients treated with different immunosuppressants. The Immunobiogram allows to stablish an individualized pattern of sensitivity/resistance for each patient.
Results: We verified the existence of different patterns of sensitivity/resistance as response to immunosuppressive treatment in 70 patients. We detected that patients who had not been treated with a specific immunosuppressant are more sensitive to such treatment than patients already treated. Moreover, we determined the existence of a correlation between the dose supplied of a certain drug during the treatment and the decrease of the sensitivity. This decrease dose-dependent sensitivity is determined at least in part by the establishment of resistance to drug, which entails to an increase in the ID50 (half-maximal inhibitory dose) necessary for the maintenance of immunosuppression.
Conclusions: When evaluating the immunological status of the patient treated with immunosuppressants, it should be considered two antagonistic variables: the loss of synergistic activation networks of the immune system due to drug effect and the generation of molecular mechanisms of resistance to treatment. The Immunobiogram will allow us monitor appearance, or not, of these resistances.

P.A3.04 Immunomonitoring and biomarkers - Part 4

P.A3.04.01

A study of hs-CRPas cardiovascular risk marker in pre-hypertension

***S. Das**;*
School of Medical Sciences and Research, Greater Noida, India.

Background: Hypertension has turned into a leading cause of non-communicable disease associated with mortality and morbidity in both developing as well as developed world. The concept of pre-hypertension, defined as a systolic blood pressure of 120-139 mmHg and/or a diastolic blood pressure of 80-89 mmHg was introduced as the new guideline for the management of blood pressure. Hs-CRP has been studied extensively as marker of cardiovascular risk, however its role as a marker of cardiovascular risk in pre-hypertension is not well defined. The aim of this study was to explore the role hs-CRP cardiovascular risk marker in patients of pre-hypertension.
Methods: 50 adult patients, above 19 years of age ,with diagnosed pre-hypertension and 50 age and sex matched healthy controls were studied in a tertiary health care center in Uttar Pradesh India, over a period of 6 months. The serum levels of hs-CRP was measured by ELISA and routine lipid profile was measured by automated analyzer. Framingham Risk scoring scoring was also done for all the patients. Data is presented as Mean±S.D. and relationships were determined by Pearson correlations.
Results : The mean age of the patients was 51±6.5 years (72% men, 28%women). The mean serum Hs-CRP levels [5.40±2.51 mg/l] for pre-hypertension were significantly higher than in controls [0.91±0.76 mg/l] [p<0.001]. Framingham Risk score was higher for patients with
pre-hypertension than controls. Higher hs-CRP values correlated with higher Framingham risk score.
Conclusion: Our results suggest that hs-CRP is a marker of Cardiovascular Risk in patients of pre-hypertension.

P.A3.04.02

Serum uromodulin, a new biomarker of renal function?

***C. Esteve Cols**[1,2], F. Graterol Torres[3], M. Navarro Díaz[3], À. Soriano Martínez[1], J. Ara del Rey[3], E. Martínez Cáceres[1,2], B. Quirant Sánchez[1,2];*
[1]Immunology Deparment Hospital Universitari Germans Trias i Pujol, Badalona, Spain, [2]Department of Cell Biology, Physiology and immunology, Universitat Autònoma de Barcelona, Bellaterra, Spain, [3]Nephrology Deparment Hospital Universitari Germans Trias i Pujol, Badalona, Spain.

Membranous GlomeruloNephritis (MGN) and IgA Nephropathy (IgAN) are the leading forms of primary glomerulonephritis. MGN is caused by autoantibodies against PhospholipaseA-2 receptor, and IgAN by partially degalactosilated IgA1 (Gd-IgA1) that induces the generation of immunocomplexes of soluble IgA receptor (CD89) and Gd-IgA1.
The glycoprotein uromodulin is synthesized exclusively in the ascending limb of loop of Henle. A decrease in serum values is a sensitive marker of low renal function.
We aim, to evaluate serum uromodulin as a biomarker of renal function in comparison to serum creatinine, renal glomerular filtrate (eGFR) and proteinuria.
A retrospective study of 46 MGN, 22 IgAN patients diagnosed by renal biopsy and 9 Healthy Subjects (HS) was performed. Clinical and pathological features were collected and analyzed according to serum uromodulin levels. Analysis of serum uromodulin was performed with uromodulin-*ELISA* kit *(Euroimmun®)*.
MGN and IgAN patients had lower levels of serum uromodulin than HD (MGN: 131 ± 74,31; IgAN: 91,79 ± 57,12; HS 224,9 ± 74,70 ng/mL). There were no differences between uromodulin levels and patients age or gender.
We stratified the patients according to histopathological features of renal biopsy. MGN patients with positive biopsy for IgG4 deposits, showed a correlation between serum uromodulin-eGFR ($p<0.0007$, $r=0.62$) and serum uromodulin-creatinine ($p<0.0565$, $r=-0.38$). The same results were observed in those IgAN patients with more severe renal biopsy ($p<0.0037$, $r=0.76$; $p<0.0002$, $r=-0.87$), respectively.
The inverse correlation observed between uromodulin levels and severity of renal biopsy suggests that uromodulin might be a prognostic biomarker of renal function, especially in IgAN patients.

P.A3.04.03

Immunological characterization of IgA Nephropathy patients

C. Esteve Cols[1,2], *F. Graterol Torres*[3], *A. Teniente Serra*[1,2], *B. Quirant Sánchez*[1,2], *J. Ara del Rey*[3], *E. Martínez Cáceres*[1,2];
[1]*Immunology Deparment Hospital Universitari Germans Trias i Pujol, Badalona, Spain,* [2]*Department of Cell Biology, Physiology and immunology, Universitat Autònoma de Barcelona, Bellaterra, Spain,* [3]*Nephrology Deparment Hospital Universitari Germans Trias i Pujol, Badalona, Spain.*

IgA Nephroapthy (IgAN) is the leading form of primary glomerulonephritis affecting glomerular mesangium, with proteinuria, hematuria, hypertension and reduction of renal glomerular filtrate. Approximately 40% of cases are related to end-stage renal failure, requiring either dialysis or renal tranplantation. The gold-standard technique for IgAN diagnosis is renal biopsy. In the last years, the better knowledge of the disease, has led several authors to describe serum biomarkers that may be useful for diagnosis and prognosis, such as levels of partially degalactosilated IgA1 (Gd-IgA1), of specific IgG against Gd-IgA1, as well as sCD89 and Gd-IgA1 immune complexes. Until now, it has not been performed and exhaustive analysis of peripheral leukocyte subpopulations and CD89 expression on monocytes.
A prospective study of 22 patients diagnosed of IgAN by renal biopsy was performed. Immunophenotype of leukocyte subpopulations and CD89 expression on three monocyte subpopulations was characterized by flow cytometry.
The immunophenotype showed that patients had a higher percentage of activated and effector memory $CD4^+$ and $CD8^+$ lymphocytes, lower percentages of B transitional lymphocytes and plasmablasts, and higher percentages of NK lymphocytes $CD56^{dim}CD16^+$and myeloid dendritic cells, than healthy subjects. In parallel, those patients with poor renal function and more sever renal biopsy had lower Mean Fluorescence Intensity (MFI) of CD89 on non-classical monocytes.
This preliminary study shows that MFI of CD89 on non-classical monocytes could be used as a prognostic biomarker of IgAN. In parallel, activated leukocyte subpopulations predominate on peripheral blood of IgAN patients, which may contribute to renal damage.

P.A3.04.04

Impaired T cell metabolism in patients with major depressive disorder

H. Hasselmann[1], ***S. Gamradt***[1], *A. Taenzer*[1], *J. Nowacki*[1], *K. Patas*[2], *C. Ramien*[2], *K. Wingenfeld*[1], *C. Otte*[1], *S. M. Gold*[1];
[1]*Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Klinik für Psychiatrie und Psychotherapie, Campus Benjamin Franklin, Berlin, Germany,* [2]*Institut für Neuroimmunologie und Multiple Sklerose (INIMS), Zentrum für Molekulare Neurobiologie, Universitätsklinikum Hamburg Eppendorf, Hamburg, Germany.*

Introduction: Major depressive disorder (MDD) is associated with an increased risk of metabolic illness and immune dysregulation. This includes an impaired cellular immunity as well as a higher risk of infections such as Epstein-Barr virus (EBV). However, the underlying pathways for this association remain poorly understood.
Material and Methods: To investigate a potential link between cellular metabolism, immune dysfunction and infections in MDD, we employed T cell metabolic phenotyping and antigen-reactive T cell enrichment (ARTE) in a cohort of 28 well-characterized, antidepressant-free MDD patients and 28 carefully matched healthy controls.
Results: Basal mitochondrial respiration, spare respiratory capacity and ATP-production were significantly reduced in T cells of MDD patients compared to matched controls. Frequency and function of antigen-specific (EBV) $CD4^+$ T cells was unaltered and no increase in immune exhaustion markers (PD-1, LAG-3, CTLA-4, Tim-3) could be detected in EBV-specific or total T cells. However, we observed a small but significant increase in the expression of KLRG1 in total $CD4^+$ T cells of MDD patients.
Conclusion: Results indicate that an impaired bioenergetic profile in T cells suggestive of mitochondrial dysfunction is associated with MDD. These alterations are unlikely to simply reflect an epiphenomenon of general immune exhaustion and may be linked to early manifestations of immune senescence.
Funding: Deutsche Forschungsgemeinschaft and National MS Society

P.A3.04.07

Evaluation of matrix metalloproteinase-3 (MMP-3) as a candidate biomarker in assessment of disease activity in Algerian rheumatoid arthritis patients.

T. Hadjout[1], *S. Salah*[1], *M. Benidir*[1], *N. Klouche*[1], *M. Djennane*[2], *A. Hamdi*[1], *H. Balaouane*[1], *N. Attal*[1];
[1]*Institut Pasteur of Algeria, Algiers, Algeria,* [2]*Tizi Ouzou Hospital, Tizi Ouzou, Algeria.*

Introduction: Matrix metalloproteinase-3 (MMP-3) is a protease induced by inflammatory cytokines in rheumatoid arthritis (RA) synovium and degrade a number of extracellular matrix components of cartilage and bone. Considering its central role in RA joint destruction, serum MMP-3 level could be a clinically useful maker for disease activity assessment. The aim of our study is to evaluate serum MMP-3 levels in RA patients compared to controls and to assess whether MMP-3 levels reflect disease activity in RA patients in correlation with inflammatory markers, erosion and autoantibodies status. **Methods:** Our study groups consisted of 116 RA patients (86% women, mean age 50±13years, mean disease duration 7±9years), 66 healthy controls and 47 control patients [33 undifferentiated connective tissue disease (UCTD) and 14 Chronic Inflammatory diseases (CID)]. MMP-3 serum measurement was based on a quantitative ELISA assay (Aeskulisa MMP-3, Aesku.Diagnostics, Germany). **Results:** Serum MMP-3 was significantly higher in sera of RA patients (49±46ng/ml) compared to healthy controls (18±14ng/ml) ($p<0.001$) and UCTD patients (17±16ng/ml) (p=0,004). There was no statistical difference with CID patients (43±58ng/ml). MMP-3 levels were increased in 56% of RA patients. This statistical increasing values were found also with positivity of CRP (p=0,02), RF (p=0,03), ACPA (p=0,02) and erosive status (p=0,009). Finally, MMP-3 levels correlated positively only with RF ($p<0,0001$; r=0,35) and ACPA rates (p=0,004, r=0,26) but not with DAS28 in RA patients. **Conclusion:** Measurement of serum MMP-3 provides a particularly useful marker of inflammatory activity in RA patients and may have a particular value in predicting the progression of erosive disease.

P.A3.04.09

Immunological effects of dimethyl fumarate in multiple sclerosis patients

M. Mansilla[1], *J. Navarro-Barriuso*[1], *S. Presas-Rodríguez*[2], *A. Teniente-Serra*[1], *B. Quirant-Sánchez*[1], *C. Ramo-Tello*[2], ***E. Martínez-Cáceres***[1];
[1]*Germans Trias i Pujol Hospital and Research Institute. Campus Can Ruti, Badalona, Spain,* [2]*Germans Trias i Pujol Hospital, Badalona, Spain.*

Dimethyl fumarate (DMF) is an oral drug for the treatment of relapsing-remitting multiple sclerosis (RRMS), with an anti-inflammatory effect. It is relatively well tolerated, but it has an important impact over several leukocyte subpopulations. Consequently, immune monitorization becomes necessary to understand the effect of DMF on the immune system and to relate these changes to the clinical outcome and potential adverse effects. **Objective:** To analyze the immunological changes induced by DMF in samples of whole blood of RRMS patients. **Methods:** Longitudinal prospective study of peripheral blood T, B, NK, monocyte and DC subpopulations using multiparametric flow cytometry in whole blood from 12 RRMS patients under DMF treatment at baseline and after 1, 3, 6 and 12 months of follow up. **Results:** The study evidenced a selective reduction of T effector, T central and B memory cells in a 52.3±14.1%, 37.3±16.1% and 54.3±20.3%, respectively, was evidenced after 12 months. In contrast, the relative prevalence of both T and B naïve subpopulations increased in a 58.4±25.6% and 47.1±31.6%, respectively. Additionally, a switch in the central memory Th2/Th1 ratio from 0.8±0.3 to 1.4±0.6 and an increase of transitional B cells in absolute number (from 5±3 to 10±5 cells/µL) were spotted. All these changes were progressive from month 1, and already significant by month 6. All results were statistically significant ($p<0.05$). **Conclusions:** The beneficial effect of DMF reducing the number of clinical relapses in MS patients seems to be related with the effect of DMF depleting effector T cells and increasing transitional B cells.

P.A3.04.10

Single nucleotide polymorphisms in relapsing-remitting multiple sclerosis in Serbian patients: no association with disease susceptibility and interferon-beta treatment response

E. Milosevic[1], *M. Markovic*[1], *V. Perovic*[1], *I. Vukovic Petrovic*[1], *V. Pravica*[1], *I. Dujmovic*[2], *S. Mesaros*[2], *J. Drulovic*[2], *M. Mostarica Stojkovic*[1], *D. Popadic*[1];
[1]*Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia,* [2]*Clinic of Neurology, Clinical Center of Serbia, Faculty of Medicine, University of Belgrade, Belgrade, Serbia.*

Therapy response varies in multiple sclerosis (MS) patients and still cannot be predicted. Interferon (IFN)-β, a first-line drug for relapsing-remitting (RR) MS is not efficient in up to 50% of treated patients. Considering autoimmune etiology of MS in prone genetic background, our objective was to analyze selected single nucleotide polymorphisms (SNPs) in genes with assumed pathogenic role as potential biomarkers of disease susceptibility and response to IFN-β therapy.
SNPs in *CSF2* (rs25882), *IL10* (rs1800871, rs3024505), *CD86* (rs1129055), *CTLA-4* (rs5742909), *IL6* (rs1800795), *TNFA* (rs361525) and *IL23R* (rs11209026) were assessed in 121 consecutive patients with RRMS starting IFN-β therapy and 250 healthy controls. Response to IFN-β was defined after two-year follow-up: patients without relapses and no progression in the Expanded Disability Status Scale (EDSS) score were considered responders (R, n= 69), while those with relapses and/or progression in disability were considered nonresponders (NR, n=52). SNPs were determined by TaqMan® SNP qPCR genotyping assays.
Genotype and allele distribution was similar in MS patients compared to healthy controls. Likewise, no significant disparity in genotype and allele frequencies between R and NR patients was found for any of the explored SNPs.
Although these SNPs are thought to affect protein function, we did not found association of their variants with susceptibility to MS and IFN-β therapy outcome, including the ones whose role in MS pathogenesis has not been addressed thus far (*CSF2* (rs25882) and *IL10* (rs3024505)).
Supported by Serbian Ministry of Education, Science and Technological development (Grant No. 175038 and 175031)

P.A3.04.11

Determination of monocytes, dendritic cells, NK cells and T regulatory cells in patients with type 1 diabetes and healthy children

***A. Oras**, R. Uibo;*
University of Tartu, Tartu, Estonia.

Introduction: Type 1 diabetes (T1D) is an autoimmune disease characterized by immune-mediated destruction of the insulin-producing β-cells in pancreatic islets. Altered numbers of innate immune cells such as dendritic cells (DC), natural killer (NK) cells and monocytes as well as adaptive immune cells such as T regulatory (Treg) cells have been demonstrated in previous studies. The aim of this ongoing study is to compare the numbers of aforementioned cell populations in children with T1D and healthy controls. **Material and Methods:** We investigated whole blood samples from patients with recent onset of T1D and healthy children. Relative numbers of DC, plasmacytoid DC, myeloid DC, monocytes, NK cells, Tregs and their subpopulations were determined based on differential expression of HLA-DR, CD16, CD56, CD14, CD123, CD11c, CD4, CD25, CD127 and CD194. For calculating the absolute cell counts BD TruCOUNT Beads were used. The data was analyzed according to the COST-ENTIRE HIP-C version 3.3 protocol and immunophenotyping of whole blood was performed on the BD LSRFortessa™. Mann-Whitney U test was employed to assess the difference in study groups. **Results:** Our studies revealed statistically significant differences in dendritic cells subsets between patients with T1D and healthy controls. **Conclusions:** Our results confirm the suitability of HIP-C 3.3 protocol for testing peripheral blood innate immunity and Treg cell subpopulations; and support the possible involvement of some of these subsets in T1D pathogenesis.

P.A3.04.12

A/professor, PhD

*D. Jain, M. O'Malley, **K. M. Paulsson**;*
Experimental Medical Science, Lund, Sweden.

Tapasin edits HLA-Imolecules and hence plays a crucial role in immunological surveillance. Our novel data shows that recombinant tapasin dissolves abberant conformations of HLA-I when added to the cell surface. During inflammation and in tumours pH is decreased. To study the effect on tapasin secondary structure at different pH and temperature we here used Synchrotron Radiation Circular Dichroism (SRCD) spectroscopy and Small Angle X-ray Scattering (SAXS) to study the dynamic solution structure of tapasin under physiological conditions. Contrary to crystal structure data, which was performed at room temperature, we found that tapasin undergoes structural transition and acquires a characteristic fold at physiological temperature i.e at 37C. Interestingly, decreased pH also resulted in the same conformational change of structure. These results show a conformational change in tapasin at low pH as well as at physiological temperature, which is highly relevant to consider when e.g, developing new peptide based vaccines or studying HLA-I antigen presentation at physiological temperature and in inflamed tissue with lower pH.

P.A3.04.13

Relationship between serum protein pattern and disease activity in patients with systemic sclerosis

***A. Petrackova**, A. Smrzova, T. Dyskova, R. Fillerova, G. Gabcova, M. Schubertova, M. Skacelova, F. Mrazek, P. Horak, E. Kriegova;*
Faculty of Medicine and Dentistry, Palacky University and Hospital Olomouc, Olomouc, Czech Republic.

Introduction: Systemic sclerosis (SSc) is a complex autoimmune connective tissue disorder with varying manifestations and clinical outcomes. There is still limited information of serum biomarkers suitable for effective monitoring of disease activity.
Methods: We investigated the serum levels of 92 inflammation-related proteins in 52 Czech patients with SSc and 24 age/gender-matched healthy control subjects using a highly sensitive innovative multiplex PEA (Olink Bioscience, Sweden). Subgroups were formed based on the disease activity (non-active SSc, n=14; active SSc, n=38), where revised EUSTAR activity index of >2.25 was taken as active SSc. Statistics were performed using GenEx (Sweden).
Results: Top-ranked proteins distinguishing SSc and healthy controls (P_{corr}<0.00001) were sTNFSF14, axin 1, sulfotransferase 1A1, CCL7, caspase 8, sTGFα, FGF23, and CXCL10. When comparing SSc patients with active and non-active disease, upregulation of IL-6, CCL7, IL-10, sTGFα, FGF21, CCL23, oncostatin M, EN-RAGE, CSF1, sHGF, GDNF, CCL11, and downregulation of sTNFβ was observed to be associated with disease activity (P<0.05). Moreover, serum levels of IL-6, CCL7, IL-10, CSF1, sHGF positively correlated (r>0.353, P<0.009) and sTNFβ negatively correlated (r=-0.374, P<0.006) with levels of C-reactive protein. Further multivariate analysis is needed to identify pattern associated with high disease activity.
Conclusions: This study nominated novel serum markers IL-6, CCL7, IL-10, CSF1, sHGF, and sTNFβ for evaluation of the disease activity of patients with SSc. Larger cohorts and multivariate analysis will be needed to prove their usefulness as biomarkers for active SSc.
Grant support: MZ CR VES15-28659A, IGA UP_2018_016, MH CZ - DRO (FNOL, 00098892)

P.A3.04.14

Phagocyte metabolic profile in different models of Parkinson's disease in rats

***M. P. Rudyk**[1], Y. Hurmach[1], V. Svyatetska[1], A. Prysiazhniuk[1], K. Stepura[1], A. Shuliak[1], Y. Maiboroda[1], T. Dovbynchuk[1], N. Khranovska[2], O. Skachkova[2], G. Tolstanova[1], L. Skivka[1];*
[1]Institute of biology and medicine, Kyiv, Ukraine, [2]National Cancer Institute, Kyiv, Ukraine.

Introduction: The local inflammation in CNS during Parkinson's disease (PD) is associated with phenotypic and functional changes not only in local microglia but also in peripheral phagocytes. The aim of this study was to compare the functional state of phagocytes from different locations in rats with MPTP-induced and 6-OHDA-induced PD. **Methods:** PD in rats was induced with subcutaneous injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or unilateral stereotaxic injection of 6-Hydroxydopamine (6-OHDA) into the striatum. Reactive oxygen species (ROS) generation and phagocytosis activity, as well as CD14, CD69, CD80/86 and CD206 expression by microglia, circulating phagocytes and peritoneal macrophages were evaluated by flow cytometry. NO production and arginase activity of the cells were examined in colorimetric assays. **Results:** MPTP-induced PD in rats was associated with increase in highly phagocytic $CD14^{high}$ CD 69+ microglia fraction. $CD14^{high}$ $CD69^{high}$ peritoneal macrophages were characterised by increased NO production. Circulating phagocytes showed upregulated CD69, while their ROS production and phagocytosis were decreased. In rats with 6-OHDA-induced PD, sharply decreased phagocytosis along with increased ROS and NO production by microglia were detected. Decreased CD14 and CD80/86 expression, along with high CD206 expression were observed on microglia of these animals. Progressive neuronal damage led to monocytosis. Circulating phagocytes and peritoneal macrophages showed pro-inflammatory activation. **Conclusion:** Inflammatory process in CNS of rats with PD was characterised by their microglia strong activation, more pronounced in case of 6-OHDA-induced PD. Peripheral phagocyte metabolic changes in rats with different PD models have different activation patterns indicating distinct disease immunopathogenesis.

P.A3.04.15

Immunomonitoring of Treg heterogenity in Multiple Sclerosis

***M. Sambucci**[1,2], F. Gargano[1], V. De Rosa[2], M. De Bardi[1], M. Picozza[1], R. Placido[1], S. Ruggieri[3,4], A. Capone[1,5], C. Gasperini[3], G. Matarese[6,2], L. Battistini[1], G. Borsellino[1];*
[1]Laboratory of Neuroimmunology, Fondazione Santa Lucia, Rome, Italy, [2]Institute of Experimental Oncology and Endocrinology, National Research Council (IEOS-CNR), Immunology Lab, Naples, Italy, [3]Department of Neurosciences, San Camillo Forlanini Hospital, Rome, Italy, [4]Department of Neurology and Psychiatry, Sapienza University of Rome, Rome, Italy, [5]Laboratory of Neuroembryology, Fondazione Santa Lucia, Rome, Italy, [6]Department of Molecular Medicine and Biotechnologies, University of Naples "Federico II", Naples, Italy.

Introduction: Regulatory T cells (Treg) are a fundamental component for immune regulation and homeostasis. In humans, Tregs are a heterogeneous population for gene expression, phenotype, and suppressive functions. While there is a general consensus regarding the surface markers required for their identification, several studies have identified distinct subsets within this cell population based on the evidence that several FoxP3 isoforms exist. The two most relevant FoxP3 isoforms are the full length isoform (FoxP3fl) and the isoform lacking exon 2. Since FoxP3 regulates the development and function of Treg cells, different FoxP3 isoforms lead to different Treg cells.
Material and Methods: Fresh PBMC (ex-vivo) from MS patients and healthy controls (HD) were stained to define the phenotype of Treg cells, using antibody clones that detect different FoxP3 isoforms. Total cell lysates were used to perform Western Blots. When the amount of cells was very low total RNA was isolated and PCR was performed.
Results: We find that Treg cells from MS patients preferentially express the Foxp3 isoform lacking exon 2. This isoform confers reduced suppressive abilities. We also find that in patients T reg cells express markers of cellular exhaustion. These findings may explain the the less effective immune regulation found in patients with autoimmune disease.
Conclusion: This study underlines the importance of studying T regulatory cells taking in consideration the existence of distinct FoxP3 isoforms which identify cells with potentially diverse suppressive abilities and which may be differently expressed in health and in disease.

P.A3.04.16

Analysis of relationship of functional activity of peripheral immune cells with the course of Parkinson's disease

J. Teterina[1], *A. Boyko*[1], *N. Troyanova*[1], *M. Grechikhina*[1], *N. Ponomareva*[2], *V. Fokin*[2], *E. Fedotova*[2], *T. Azhikina*[1], *E. Kovalenko*[1], *A. Sapozhnikov*[1];
[1]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russian Federation,* [2]*Federal Research Organization "Scientific Centre of Neurology", Moscow, Russian Federation.*

Parkinson's disease (PD) is one of the most common neurodegenerative diseases. In recent years, there is growing evidence that the pathogenesis of this disease is connected with regional and peripheral immune processes. Currently, the association of clinical signs of PD with different characteristics of patient immune status is actively being searched. In the framework of this problem we perform an investigation of functional state of immune cells, in particular, activity of their chaperone-associated system of protein homeostasis, in patients with PD. 26 healthy donors and 21 patients with PD were examined during the first stage of the study. Flow cytometric analysis of intracellular HSP70 in populations of granulocytes (PMN) and mononuclear cells (PBMC) in normal and heat shock conditions did not reveal significant distinctions between the groups of healthy volunteers and the patients. However, analysis of basal transcription activity of different HSPA genes demonstrated a considerable increased expression of minor stress-induced HSPA6 gene in PBMC fractions of the patients with PD as compared to the healthy donors. The data suggest that PD dose not associate with essential alterations of HSP70 synthesis in peripheral blood leukocytes. However, the course of PD might be accompanied by increased transcription of some stress-induced HSPA genes in peripheral immunocompetent cells. This work was supported by Russian Science Foundation, grant No. 16-15-10404.

P.A3.04.17

Serum cytokines and chemokines in newly diagnosed Ulcerative Colitis patients predicting the effect on treatment and disease course.

E. G. van Lochem[1], *B. Roosenboom*[1], *C. Smids*[1], *P. Wahab*[1], *M. Groenen*[1], *S. Nierkens*[2], *E. van Koolwijk*[1], *C. Horjus Talabur Horje*[1];
[1]*Rijnstate Hospital, Velp, Netherlands,* [2]*Utrecht Medical Center, Utrecht, Netherlands.*

Predicting disease course and treatment response remains difficult in patients with Ulcerative colitis (UC). Based on our previous study on Crohns disease and new insights in UC pathophysiology, we identified distinctive serum analytes associated with disease activity and course in newly diagnosed, untreated patients at presentation and during follow-up. Multiplex-immunoassay analysis for 21 cytokines, chemokines and receptors was performed on blood samples from 45 UC patients at diagnosis and follow-up (n=20) and compared to healthy controls (n=18, HC). Results: Compared to HC, baseline UC patients had significant higher levels of IL-6, IL-7, IL-17, Oncostatin, CCL-11, CCL-19, CXCL-1, CXCL-13, MMP-10, sTNF-R2 and sIL2RsCD25. Comparing mild endoscopic disease (Mayo 1, N=14) with severe endoscopic disease (Mayo 3, N=9), higher serum levels of IL-6 (p=0.001), IL-17 (p=0.019), OncostatinM (p=0.002), CXCL-1 (p=0.011), GCSF (p=0.001) and sTNFR-2 (p=0.009) were found in patients with severe disease.
The levels of IL-6 (p=0.038), IL-7 (p=0.028) and MMP-10 (p=0.038) in UC patients during remission, dropped significantly to the level of HC (n=9) after follow-up. Patients who did not respond on corticosteroids (n=5) had higher levels of IL-10 (p=0.001) and IL-13 (p=0.005) at baseline; non-responders on anti-TNF treatment (n=3) had higher IL-10 (p=0.006) levels. Patients with an exacerbation within one year had significant higher IL-10, OncostatinM, CXCL-8, GCSF and sTNFR-2 levels at baseline compared to patients without exacerbation. In this cohort of newly diagnosed UC patients we identified IL-6 as a possible predictor of endoscopic disease severity. Higher IL-10 and IL-13 levels at baseline were associated with non-responding on corticosteroids.

P.A3.04.18

SERUM ANGIOGENESIS MARKERS PREDICT DISEASE OUTCOME IN GIANT CELL ARTERITIS

Y. van Sleen, *M. Sandovici, W. H. Abdulahad, J. Bijzet, A. M. Boots, E. Brouwer;*
University of Groningen, University Medical Center Groningen, Groningen, Netherlands.

Giant cell arteritis (GCA) is an inflammatory large vessel disease requiring long-term treatment with glucocorticoids. Relapses occur often and the high cumulative glucocorticoid dose has detrimental effects on patient's quality of life. Therefore, new biomarkers are needed for diagnosis and disease course prediction. As the majority of infiltrating cells in GCA vessels are macrophages, the aim of this project was to evaluate levels of macrophage serum products in GCA patients as candidate disease course predictors.
Forty-one newly diagnosed GCA patients were recruited before start of glucocorticoids. Disease course was monitored and time to glucocorticoid treatment-free remission was prospectively documented. Also, thirty age- and sex-matched healthy controls (HCs) were included. Serum concentrations of IL-6, SAA, sCD163, calprotectin, YKL-40, and angiopoietin-1 and 2 were determined by ELISA or Luminex assay at baseline, during treatment and in treatment-free remission.
IL-6, SAA, sCD163, calprotectin, YKL-40, VEGF and angiopoietin-2 levels were elevated in GCA compared to HCs. High VEGF and angiopoietin-1, but low YKL40 and angiopoietin-2 levels, at baseline predicted a shorter time to treatment-free remission. Other markers had no predictive value. In patients on treatment, angiopoietin-2 levels were increased shortly before relapse. Patients in treatment-free remission still showed increased levels of angiopoietin-1 and 2, sCD163, IL-6 and calprotectin compared to healthy controls. Markers of angiogenesis hold promise as predictors of disease outcome at baseline and during treatment. Validation of this angiogenesis signature in independent cohorts is required. Whether patients in treatment-free remission may still suffer from smouldering vessel wall inflammation needs further investigation.

P.A3.04.20

Circadian rhythm of immune cells in healthy individuals and patients with rheumatoid arthritis

S. Wilantri[1,2], *C. Strehl*[1,2], *D. Abdirama*[1,2], *T. Gaber*[1,2], *F. Buttgereit*[1,2];
[1]*Charité Universitätsmedizin Berlin, Berlin, Germany,* [2]*Deutsches Rheuma Forschungszentrum, Berlin, Germany.*

Circadian rhythms have been appreciated as important regulators of various biological processes. Disrupted circadian rhythms were observed in patients suffered from cancer, tumor and inflammatory diseases. Our previous 24-hour pilot study suggested that immunological circadian rhythms in patients with rheumatoid arthritis (RA) were altered compared to the healthy individuals. Fourteen eligible female patients with RA aged 45-75 and twelve eligible healthy individuals were recruited to join the study. The circadian rhythms were synchronized by scheduled food and water intake and sleeping period a week prior to study day. On the study day, the blood was drawn in two hours interval throughout 24 hours. The absolute number of circulating immune cells was determined by TruCount. RNA was isolated from CD14+ monocytes and analyzed by qPCR.We investigated major immune populations and found striking differences in the circadian rhythm of NK T cells, NK cells, CD8 T cells, CD3 T cells, CD4 T cells and regulatory T cells of healthy individuals and patients with RA. Phase shifting, reduction of amplitude, attenuation and new establishment of circadian rhythms were observed in patients with RA. In addition, the qPCR data also indicated a disrupted circadian rhythm in RNA level. Among ten clock genes that were examined, REVERBα, PER1, and PER3 showed altered rhythmic expression in patients with RA. We will continue to investigate the circadian rhythms using mass cytometry and multiplex immunoassay. Identification of circadian rhythm in healthy individuals and patients with RA may provide an outlook for future implication, such as in chronotherapy.

P.A3.04.21

The Structure and Functional regulation of CD36 and Its Role in Atherosclerosis

K. Zhang, *L. Peng, H. Wei;*
Department of Laboratory Medicine, the Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing University, Nanjing, PR China, Nanjing, China.

CD36 is a multifunctional transmembrane glycoprotein that binds to oxidized low density lipoprotein to induce monocyte transformation into foam cells and promotes the formation of atherosclerosis through inflammatory response, oxidative stress, platelet activation, macrophage trapping. Inhibition of CD36 expression or interference with its associated signaling pathways can significantly alleviate the severity of atherosclerosis. In addition, the highly expression of CD36 in the tongue, nasal cavity, small intestine and brain promotes the body's intake and absorption of lipids, and increases the risk factors of metabolic diseases. Serum soluble CD36 is found as a component of circulating microparticles (MPs) and may be as a predictor for atherosclerotic disease.

P.A3.04.22

Retrospective study of autoantibodies control at patients in hospital at Northwesrern Greece

N. Zotos[1], *P. Christodoulou*[1], *E. Tatsina*[2], *L. Papageorgiou*[1], *N. Varsamis*[1], *G. Katagis*[1], *E. Mosheta*[1], *M. Gerasimou*[1], *A. Zotou*[1], *D. Bougias*[1], *M. Gianniki*[3], *A. Pournou*[1], *N. Tsifetaki*[1];
[1]*General Hospital of Ioannina, Greece, Ioannina, Greece,* [2]*Papageorgiou Hospital, Thessaloniki, Greece,* [3]*Agia Sofia Hospital, Athens, Greece.*

Introduction: Positive anti-nuclear antibodies are detected with high frequency in systemic rheumatic diseases, infections, inflammations, neoplasms as well as in normal individuals. Detection and identification of autoantibodies significantly help in accurate diagnosis, prognosis, treatment regulation, and clinical follow-up of patients. Aim: The analysis of the antinuclear antibody (ANA) detection results and the association of positive ANAs with the occurrence of specific antinuclear antibodies: anti-double stranded DNA (anti-dsDNA) and anti-extrapolated core antigens (ENA) in patients both internals and externals. Material-Method: A total of 383 patients (126 internal and 257 external) were examined over a 12-month period from January 2017 to December 2017. The measurement of autoantibodies was performed by indirect immunofluorescence. Results: A total of 28 subjects (7.31%) with positive anti-nuclear antibody titles, 25 women and 3 men were found. Among the negative results, 29 serum boundary sera were found. Of the 28 ANA-positive sera, 9 were found with anti-ENA (+), 2 showed anti-dsDNA (+), and 6 subjects were found positive with anti-ENA and anti-dsDNA. The remaining 11 individuals (2.87%) with ANA positive had specific anti-nuclear antibodies negative. Of the 29 marginal activity sera, 6 showed anti-ENA (+) and one sample with a known SLE history showed a high anti-dsDNA title. Conclusions: ANAs were detected in 7.31% of patients, while 2.87% of the total number of patients had ANA (+) without specific individual antibodies. The evaluation of both a negative and a positive result should be done with great care.

P.A3.04.23

Electrochemical biosensing of cancer exosomes in human serum based on magnetic separation

*S. Lima de Moura[1], M. Pividori[1], **M. Martí**[2];*
[1]Grup Sensors i Biosensors. Dpt Química. Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain, [2]Institut de Biotecnologia i Biomedicina, Bellaterra (Barcelona), Spain.

The identification of novel biomarkers represents a worldwide challenge not only for the improvement of early diagnostics, but also for patient monitoring and for the evaluation of the efficiency of a therapeutic strategy. Exosomes are nano-sized and cup-shaped vesicles, which are currently under intensive study as potential diagnostic biomarkers for many health disorders, including cancer. Therefore, this is a growing need for sensitive methods capable of accurately and specifically determining the concentration of exosomes. This work addresses the study of different receptor by flow cytometry as well as the design of a quantitative and rapid method for total exosome counting based on magneto-actuated platforms with electrochemical readout. Two different strategies were explored for the magnetic separation of exosomes. Briefly, based on i) the direct covalent immobilization on tosyl-activated magnetic particles or, instead, by ii) immunomagnetic separation based on different receptors. The magneto electrochemical biosensor for the exosomes counting was successfully achieved in human serum. This proof-of-concept device represents a rapid, cost-effective, and high-sample-throughput detection of exosomes and can be potentially established as promising approach for cancer diagnostics based on liquid biopsy.

P.A3.04.24

Magneto-actuated rapid test for the detection of circulating tumor cells

*A. Pallarès[1], S. Lima de Moura[1], M. Mesas[1], **M. Martí**[2], M. Pividori[1];*
[1]Grup Sensors i Biosensors. Dpt Química. Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain, [2]Institut de Biotecnologia i Biomedicina, Bellaterra (Barcelona), Spain.

According to WHO, breast cancer is the top cancer in women both in the developed and the developing world, and the number of new cases is expected to rise by about 70% over the next two decades. The early and accurate detection of breast cancer as well as the risk of metastasis in small healthcare centers remains as the cornerstone of breast cancer control. This work is intended to contribute in the development of Rapid Diagnostic Test (RDTs) for cancer diagnosis at point-of-care in low resource settings, taking breast cancer circulating tumor cells from MCF7 cellular line as a model. Two different strategies were designed: a magneto-actuated immunosensor for the quantification of the breast cancer cells and a magnetic genosensor for the detection of the PCR-amplified genetic material from the cells. For that purpose, different commercial antibodies against specific epitopes of the cellular membrane were firstly studied by flow cytometry and confocal microscopy.
Such antibodies were then covalently immobilized on magnetic particles to capture the tumor cells by immunomagnetic separation for the preconcentration of the cells from complex samples and immunosensing with an specific antibody. The magneto genosensing approach is based on a double-tagging RT-PCR amplification of the transcripts from the cells and the quantification of the amplicon by amperometry technique or visual readout based on lateral flow. Finally, the results of these strategies are compared in terms of the analytical performance, showing promising features for being used as RDTs.

P.A3.05 Immunomonitoring and biomarkers - Part 5

P.A3.05.01

Innate immune recovery predicts CD4+ T-cell reconstitution after hematopoietic cell transplantation

***C. de Koning**[1], J. Langenhorst[1], C. van Kesteren[1], C. A. Lindemans[2], A. Huitema[1], S. Nierkens[1], J. J. Boelens[2,1];*
[1]University Medical Centre Utrecht, Utrecht, Netherlands, [2]Wilhemina Children's Hospital, Utrecht, Netherlands.

Innate immune cells are the first to recover after allogeneic hematopoietic cell transplantation (HCT). Nevertheless, reports of innate immune cell recovery and their relation to adaptive recovery after HCT, are largely lacking. Especially predicting CD4+ T-cell reconstitution is of clinical interest, as this parameter directly associates with survival chances after HCT.
We developed a multivariate, combined non-linear mixed-effects model for monocytes, neutrophils and NK-cell recovery after transplantation. We evaluated whether innate recovery relates to CD4+ T-cell reconstitution probability, and investigated differences between innate recovery after cord blood transplantation (CBT) and bone marrow transplantation (BMT).
205 Patients, undergoing a first HCT (76 BMT, 129 CBT) between 2007-2016, were included. The median age was 7.3 years (range 0.16-23). Innate recovery was highly associated with CD4+ T-cell reconstitution probability (p<0.001) in multivariate analysis correcting for covariates. Monocyte (p<0.001), neutrophil (p<0.001), and NK-cell (p<0.001) recovery reached higher levels during the first 200 days after CBT compared to BMT. The higher innate recovery after CBT may be explained by increased proliferation capacity (measured by Ki-67 expression) of innate cells in CB-grafts compared to BM-grafts (p=0.041), and of innate cells in vivo after CBT compared to BMT (p=0.048). At an individual level, patients with increased innate recovery after either CBT or BMT had received grafts with higher proliferating innate cells (CB; p=0.004, BM; p=0.01, respectively).
Our findings implicate the use of early innate immune monitoring to predict the chance of CD4+ T-cell reconstitution after HCT, with respect to higher innate recovery after CBT compared to BMT.

P.A3.05.02

Effects of adalimumab on T-helper-17 lymphocyte and neutrophil related inflammatory serum markers in patients with moderate to severe hidradenitis suppurativa

***R. de la Varga Martínez**[1,2], D. Jiménez Gallo[3], L. Ossorio García[3], M. Linares Barrios[3], C. Rodríguez[2];*
[1]Servicio de Inmunología, UGC de Laboratorios Clínicos. Hospital Universitario Virgen del Rocío, Sevilla, Spain, [2]Servicio de Inmunología, UGC de Hematología, Inmunología y Genética. Hospital Universitario Puerta del Mar, Cádiz, Spain, [3]UGC de Dermatología. Hospital Universitario Puerta del Mar, Cádiz, Spain.

Introduction: T-helper (Th)-17 lymphocytes and neutrophils are the main sources of the proinflammatory cytokines involved in the pathogenesis of hidradenitis suppurativa (HS). Objective: This study aims to evaluate the improvement of the inflammatory serum markers (ISM) levels in patients with moderate-to-severe HS who receive adalimumab. Methods: Nineteen moderate-to-severe HS patients were prospectively recruited. Each of the patients received 40 mg of adalimumab weekly. The ISM levels and modified Hidradenitis Suppurativa Score (mHSS) scores were assessed at baseline and at week 36. Nineteen healthy volunteers (HC) constituted the control group. Results: Before adalimumab treatment, the HS patients showed significantly increased levels of interleukin (IL)-6, IL-8, IL-10, IL-17A, soluble TNF receptor II (sTNF-RII), and C-reactive protein (CRP) as well as an increased erythrocyte sedimentation rate (ESR) (all p<0.01). At week 36, the circulating levels of IL-1β, IL-6, IL-8, IL-10, IL-17A, soluble TNF receptor I (sTNF-RI), sTNF-RII, and CRP, as well as the ESR (all p<0.05), decreased significantly in the HS patients who received adalimumab. The decrease in levels of IL-6 (r=0.65, p=0.003), IL-8 (r=0.52, p=0.024), sTNF-RI (r=0.55, p=0.015), and CRP (r=0.47, p<0.040) and the ESR (r=0.60, p<0.006) were significantly well correlated with clinical improvements according to the mHSS. Conclusions: Adalimumab improves the ISM-based systemic inflammatory burden in patients with moderate-to severe HS. IL-6, IL-8, sTNF-RI and CRP and the ESR may serve as novel biomarkers for a therapeutic response.

P.A3.05.03

Characterization and enumeration of immune phenotypes in individuals from different age groups

***M. de Zeeuw-Brouwer**[1], L. de Rond[1], L. Samson[1], D. van Baarle[1,2], F. van der Klis[1], A. M. Buisman[1];*
[1]National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands, [2]University of Utrecht, University Medical Centre Utrecht (UMCU), Utrecht, Netherlands.

Background Ageing is accompanied by alterations of the immune system and often leads to a decline in immune function. To assess the level of variation in immune phenotype of individuals with age, different lymphocyte subsets were analysed in a cross-sectional population study, performed in 2016/2017. Method Extensive whole blood immunophenotyping of absolute numbers of several immune subsets and their activation markers, was performed in 4 age groups (5-8 yr, 18-25 yr, 40-45 yr and 65-70 yr, n=60-90) Results No differences in the absolute numbers of lymphocytes, monocytes and granulocytes among the age groups were observed. Not surprisingly, children (5-8 yr) had significantly more (naïve) B-cells than other age groups. The numbers of $CD56^{bright}CD16^{dim/-}$ NK cells and CD4+HLADR-CD38+ cells were also elevated in children, while numbers of $CD56^{dim}CD16^{+}$ NK cells were lower. No differences were observed in numbers of CD4 cells, while a decline in those of CD8 cells was seen with increasing age. Also the amount of CD4+HLADR+CD38- T-cells increased during ageing. Conclusion Most differences in numbers of lymphocyte subsets were seen in children (5-8 yr), compared to other age groups. Especially numbers of CD4+HLADR-CD38+ and $CD56^{bright}CD16^{dim/-}$ cells were high in children, which indicates more naïve/immature T- and NK cells in this age group. A higher level of CD4+HLADR+CD38- cells in the older groups suggests the presence of more activated memory T-cells with ageing. These data will be combined with functional immunological assays, to get more insight in alterations of the various immune subsets during ageing.

P.A3.05.04

T helper cell subsets and related cytokines in infertile women undergoing in vitro fertilization before and after seminal plasma exposure

*M. Azad[1], S. Keshtgar[2], Z. Kanannejad[1], B. Namavar-Jahromi[3,4], **B. Gharesi-Fard**[1,4];*
[1]Department of immunology, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of, [2]Department of Physiology, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of, [3]Department of Obstetrics and Gynecology, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of, [4]Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of.

In vitro fertilization (IVF) is a well-known method for the treatment of infertility. The present study aimed to compare the differences between infertile women with successful and unsuccessful IVF outcomes regarding the expression of T helper (Th) cell transcription factors and a group of related cytokines before and after exposure to their husbands' seminal plasma. This study was performed on 19 couples with unexplained infertility undergoing IVF treatment. Among the studied group, nine and 10 couples had successful and unsuccessful IVF outcomes, respectively. This study was carried out using real-time polymerase chain reaction. Before seminal plasma exposure, the expression levels of T-bet (p<0.007), interferon-γ (p=0.013), and TNF-α (p=0.017) were higher in the infertile women with IVF failure than in those with successful IVF outcomes, while those of GATA3 (p<0.001), Foxp3 (p=0.001), and interleukin (IL)-35 (p<0.003) were lower. After seminal exposure, the expression of T-bet (p=0.02), Rorc (p<0.001), TNF-α (p=0.001), Foxp3 (p=0.02), and interferon-γ (p=0.001) increased in the unsuccessful IVF group, while the expression of Foxp3 (p=0.02), Rorc (p<0.001), IL-23 (p=0.04), IL-17 (p=0.02), IL-6 (p<0.001), transforming growth factor-β (p=0.01), and IL-35 (p<0.001) increased in the successful IVF group. In summary, IVF failure was associated with imbalanced Th1/Th2/Th17/Treg responses. Moreover, our results show that seminal plasma might have a positive effect on IVF outcomes via changes in peripheral blood T cell subsets.

P.A3.05.05

Difference in protein expression of the peripheral blood CD4+ T lymphocytes between polycystic ovary syndrome and healthy women

***B. Gharesi-Fard**[1,2], F. Nasri[1];*
[1]Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of, [2]Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of.

Proteome profile analysis of CD4+ T lymphocytes in polycystic ovary syndrome (PCOS) may represent the proteins involved in the pathogenesis of the disease. The present study aimed to compare the protein expression profiles of the peripheral blood CD4+ T lymphocytes between PCOS patients and healthy women. We used two-dimensional gel electrophoresis (2-DE) followed by mass spectrometry (MS) of selected protein spots. Moreover, identified protein spots were confirmed by western blot technique. Despite the overall proteome similarities between patients and healthy women, the analysis of protein spots revealed that at least seven spots were differently expressed (P< 0.05). Protein identification was successfully achieved for 3 out of 7 spots by Mass technique and confirmed by western blot. All 3 identified proteins including Phosphatidyl ethanolamine-binding protein 1(PEBP1), Proteasome activator complex subunit 1 (PSME1), and Triosephosphate isomerase 1 (TPI) showed over-expression in PCOS patients compared with the healthy subjects. These deferentially expressed proteins might be involved in oxidative processes and cardiac pathology. This evidence highlights T lymphocytes competence as a living biosensor system to record the alteration of metabolism and gene expression and would be a good substitution for tissue biopsies.

P.A3.05.06

Expression levels of serum lncRNA HOTAIR in patients with rheumatoid arthritis

***X. Li**, S. Song, J. Yu, A. Li, F. Chen, N. Sun, Y. Yang;*
Department of Clinical Laboratory Science,Jinling Hospital, School of Medicine, Nanjing University, Nanjing, China.

Objective To investigate the different expression levels of lncRNA HOTAIR in serum of patients with rheumatoid arthritis (RA) . **Methods** 205 serum samples were selected from 95 patients with RA, 50 patients with Systemic lupus erythematosus (SLE) and 60 healthy controls. The total RNAs from serum were extracted,and the expressions of lncRNA HOTAIR, XIST and H19 were detected by the method of RT-qPCR. Pearson correlation analysis was used to explore the correlation between disease activity index(DAS28)of RA and the Expression level of HOTAIR. **Results** Compared with control and SLE groups, the expression level of HOTAIR in RA group is obviously increased(P<0.05). There were no any significant different expressions of XIST and H19 between RA and control groups. Furthermore, the serum level of HOTAIR has a positive correlation with DAS28 score (P<0.02,R^2=0.2438) and the serum level of HOTAIR in the higher activity of RA group whose DAS28 score is greater than 5.1 is higher than the activity of RA group whose DAS28 score is greater than 3.2. No significant correlations were found between levels of HOTAIR and other laboratory parameters including rheumatoid factor (RF), anti-cyclic citrullinated peptide autoantibodies, C-reactive protein (CRP), and erythrocyte sedimentation rate(ESR). **Conclusion:**Expression level of lncRNA HOTAIR in the serum of RA patients was significantly higher than healthy donors and SLE patients. HOTAIR in the serum may serve as a novel and valuable biomarker for the diagnosis of RA.

P.A3.05.07

Ligands for inhibitory and activating NK cell receptors in childhood B- and T-ALL

***M. V. Martinez-Sanchez**[1,2], L. Gimeno-Arias[1,2], J. F. Pascual-Gázquez[3,2], A. M. Fita[3,2], E. Ramos-Elbal[3,2], A. M. Galera-Miñarro[3,2], M. M. Bermúdez-Cortés[3,2], M. E. Llinares-Riestra[3,2], M. Blanquer-Blanquer[4,2], J. L. Fuster-Soler[3,2], A. Minguela-Puras[1,2];*
[1]Immunology Service, Virgen Arrixaca Clinical University Hospital, El Palmar-Murcia, Spain, [2]Murcia BioHealth Research Institute (IMIB), Murcia, Spain, [3]Pediatric Oncohematology Department, Pediatric Service, Virgen Arrixaca Clinical University Hospital, El Palmar-Murcia, Spain, [4]Hematology and Hemotherapy Department, Virgen Arrixaca Clinical University Hospital, El Palmar-Murcia, Spain.

The level of HLA-I molecules expressed by leukemic cells is one of the most important factors influencing NK-mediated lysis. Efficient lysis requires interaction of additional activating receptors with their ligands on tumour cells such as CD112 and CD155 for DNAM-1, and MICA/B and ULBPs for NKG2D. It has been described that HLA-I down regulation is more frequent in myeloid than in lymphoblastic leukaemias and that expression of CD112 and CD155 is consistent in myeloid leukaemias but MICA/B and ULBPs were either absent or weakly expressed.
The expression of ligands for inhibitory (total HLA-I and HLA-C) and for activating (CD112, CD155, MICA/B and ULBP-1) receptors was evaluated in 45 paediatric patients at diagnosis by flow cytometry, both on bone marrow tumour cells and on normal lymphocytes as a control.
Higher expression of CD112 as percentage and as mean fluorescence intensity (MFI) was detected for B-ALL blast cells than for normal lymphocytes and higher number of CD155+ cells was observed in T-ALL blast cells than in normal lymphocytes. No differences in the expression of HLA-I and slight reduction in HLA-C were detected for B ALL blast cells compared to normal lymphocytes. A lower percentage of HLA-C cells was detected in T-ALL and lower expression of both HLA-I and HLA-C were observed in T-ALL blasts cells than in normal lymphocytes.
Differential expression of ligands for NK cells activating and inhibitory receptors in B- and T-ALL childhood blast could condition NK cell antitumor response and should be taken in consideration in NK immunotherapy protocols.

P.A3.05.08

Ligands for inhibitory and activating NK cell receptors in childhood M7 and non-M7 AML

***M. V. Martínez-Sánchez**[1,2], L. Gimeno-Arias[1,2], J. F. Pascual-Gázquez[3,4], A. M. Fita[3,2], E. Ramos-Elbal[3,2], A. M. Galera-Miñarro[3,2], M. M. Bermúdez-Cortés[3,2], M. E. Llinares-Riestra[3,2], M. Blanquer-Blanquer[5,2], J. L. Fuster-Soler[3,2], A. Minguela-Puras[1,2];*
[1]Immunology Service. Virgen Arrixaca Clinical University Hospital, El Palmar-Murcia, Spain, [2]Murcia BioHealth Research Institute (IMIB), Murcia, Spain, [3]Pediatric Oncohematology Department, Pediatric Service, Virgen Arrixaca Clinical University Hospital, El Palmar-Murcia, Spain, [4]Murcia BioHealth Research Institute (IMIB), Murcia, Saint Martin, [5]Hematology and Hemotherapy Department, Virgen Arrixaca Clinical University Hospital, El Palmar-Murcia, Spain.

The level of HLA-I molecules expressed by leukemic cells is one of the most important factors influencing NK-mediated lysis. Efficient lysis requires interaction of additional activating receptors with their ligands on tumour cells such as CD112 and CD155 for DNAM-1, and MICA/B and ULBPs for NKG2D. It has been described that HLA-I down regulation is more frequent in myeloid than in lymphoblastic leukaemias and that expression of CD112 and CD155 is consistent in myeloid leukaemias but MICA/B and ULBPs were either absent or weakly expressed. The expression of ligands for inhibitory (total HLA-I and HLA-C) and for activating (CD112, CD155, MICA/B and ULBP-1) receptors was evaluated in 9 paediatric patients at diagnosis (4 AML-M7 not related to Down syndrome and 5 AML-non-M7) by flow cytometry, both on bone marrow tumour cells and on normal granulocytes as a control. The expression of CD112 was significantly higher in AML blasts both as percentage as MFI in M7-AMLs and non-M7-AMLs compared to normal granulocytes. Increased no-significant expression for CD155 was observed in blasts compared to granulocytes. In contrast, MICA/B and ULBP were weakly expressed in blast cells and normal granulocytes. Higher expression of HLA-I in M7-AML blasts but not in non-M7-AML blasts than in normal granulocytes was observed. No significant differences were observed for HLA-C expression. Differential expression of ligands for NK cells activating and inhibitory receptors in M7 and non-M7 childhood AML blast could condition NK cell antitumor response and should be taken in consideration in NK immunotherapy protocols.

P.A3.05.09

NK cells activity and extracellular microvesicles at healthy pregnancy and preeclampsia

***V. A. Mikhailova**, K. L. Belyakova, D. O. Bazhenov, A. R. Sheveleva, E. V. Khokhlova, O. N. Arzhanova, S. A. Selkov, D. I. Sokolov;*
FSBSI "The Research Institute of Obstetrics, Gynecology and Reproductology named after D.O.Ott", Saint-Petersburg, Russian Federation.

Introduction: Pregnancy is associated with alterations in leucocytes functional characteristics. The aim was to assess functional activity of NK cells at healthy pregnancy and preeclampsia. Materials and methods: The groups included nonpregnant women, healthy pregnant women, pregnant women with preeclampsia. For assessment of NK cells cytotoxic activity we used peripheral blood mononuclear cells. For analysis of microvesicles content we used blood plasma. For confirmation the ability of NK cells to form microvesicles cell line NK-92 was used. For analysis of microvesicles influence on cells we used monocytes of cell line THP-1 and blood plasma microvesicles. The methods included cell culturing, conventional flow cytometry, high-precision flow cytometry. Results: Preeclampsia comparing with healthy pregnancy was associated with less amount of CD107a+NK cells but higher content of TRAIL+NK cells. NK cells of NK-92 line were able to form microvesicles different in phenotype. Higher counts of NK cells microvesicles with phenotype CD45+CD16+CD56+ and lower counts of CD45+CD16-CD56+NK cells microvesicles were detected in pregnant women comparing with nonpregnant. At preeclampsia there was higher content of CD45+CD16+CD56-microvesicles comparing to healthy pregnancy. The expression of CD18 and CD54 by THP-1 cells was higher after treatment with microvesicles of healthy pregnant women comparing to nonpregnant and lower after treatment with microvesicles of women with preeclampsia comparing to heathy pregnant. Conclusions: The mechanism of cytotoxity induction of NK cells, the content of NK cells microvesicles and their influence on cells differs at preeclampsia comparing to healthy pregnancy. Funding: RSF grant 17-15-01230, President's grant NSh-2873.2018.7, State program № AAAA-A18-118011020016-9.

P.A3.05.10

NK cells cytotoxity towards trophoblast cells at healthy pregnancy and recurrent pregnancy loss

***V. A. Mikhailova**, D. O. Bazhenov, L. P. Viazmina, E. V. Khokhlova, K. L. Belyakova, A. R. Sheveleva, S. A. Selkov, D. I. Sokolov;*
FSBSI "The Research Institute of Obstetrics, Gynecology and Reproductology named after D.O.Ott", Saint-Petersburg, Russian Federation.

Introduction: NK cells are present in decidua during pregnancy and can interact with trophoblast cells. The aim was to assess cytotoxic activity of NK cells towards trophoblast cells at heathy pregnancy and recurrent pregnancy loss (RPL). Materials and methods: The groups included healthy nonpregnant fertile women at proliferative (PrPh) and secretory (SecPh) phases of menstrual cycle, healthy pregnant women at 6-7 weeks of gestation (wg), nonpregnant women with RPL at PrPh and SecPh. Trophoblast cells of JEG-3 cell line were treated with CFSE (4μM). Peripheral blood mononuclear cells which contained NK cells were incubated with trophoblast cells for 4 hours. Then cell mixtures were treated with propidium iodide (0,01mg/ml). Dead trophoblast cells were detected using flow cytometer. Results: The cytotoxic activity of NK cells of fertile women in PrPh towards trophoblast cells was higher than of fertile women in SecPh and at healthy pregnancy. There was no difference in NK cells activity of fertile women in SecPh and at healthy pregnancy. The cytotoxic activity of NK cells of nonpregnant women with RPL in SecPh was higher than in PrPh and was higher than activity of NK cells of fertile women in SecPh. Conclusions: The cytotoxic activity of NK cells of fertile women towards trophoblast cells is low in SecPh that contributes occurrence of blastocyst implantation and promotes healthy pregnancy. In RPL NK cells cytotoxic activity in SecPh is high that contributes pregnancy loss in case of possible blastocyst implantation. Funding: President's grant NSh-2873.2018.7, President's scholarship SP-2836.2018.4, State program № AAAA-A18-118011020016-9

P.A3.05.11

T helper cells profile and $CD4^+CD25^+Foxp3^+$ regulatory T cells in Polycystic Ovary Syndrome (PCOS)

***F. Nasri**[1], M. Doroudchi[1], B. Namavar-Jahromi[2,3], B. Gharesi-Fard[1,3];*
[1]Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of, [2]Department of Obstetrics and Gynecology, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of, [3]Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of.

Polycystic ovary syndrome (PCOS) is considered as the most common cause of female infertility that affects 4-10% of women in the reproductive ages. Previous studies showed that T helper cell balances play an important role in successful pregnancy. Therefore, the aim of this study was to investigate the Th1/Th2/Th17/Treg paradigms in peripheral blood of infertile women with PCOS compared with healthy fertile women.
Peripheral blood mononuclear cells (PBMCs) were isolated at the late follicular phase from 10 women complicated with PCOS and 10 healthy fertile women. PBMCs were stimulated with PMA and Ionomycin in the presence of Berefeldin A as Golgi stop agent to detect intracellular cytokine production (IFN-γ, Il-17, and IL-4) from $CD3^+CD4^+$T cells population indicating T helper (Th) cells subsets by flowcytometry technique. Moreover, regulatory T cells were checked using CD25 and Foxp3 markers.
Results indicated that T helper cells type 1 (Th1) were statistically increased over Th2 in infertile PCOS groups when considering Th1/Th2 ratio (P=0.05) Moreover analysis of Th17/Th2 ratio showed a significant difference with a bias toward Th17 dominancy (P=0.02) for PCOS women. Finally the proportion of $CD4^+CD25^+Foxp3^+$ regulatory T cells significantly was decreased in PCOS patients as compared with that of healthy fertile women (P=0.02).
In summary results of the present study showed that over-activation of Th1 and Th17, as inflammatory subsets, and reduction of Treg and Th2, as regulators of inflammation, might be one of the underlying mechanisms in the pathogenesis of PCOS patients.

P.A3.05.13

Development of frailty is associated with elevated CRP trajectories and increased numbers of innate immune cells

***L. D. Samson**[1,2,3], P. Engelfriet[1], W. M. Verschuren[1], A. M. Boots[3], A. Buisman[2];*
[1]Center for Nutrition, Prevention and Health Services, National Institute of Public Health and the Environment, Bilthoven, Netherlands, [2]Center for Infectious Disease Control, National Institute of Public Health and the Environment, Bilthoven, Netherlands, [3]Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands.

With age, the immune system undergoes several changes such as shift from naive to memory T cells, thymic involution and increase in inflammatory cytokines. However, clinical relevance of these changes is relatively unknown. The longitudinal Doetinchem Cohort Study (DCS) (n=±3700), wherein health parameters and blood samples have been collected every five years since 1987, provides a unique framework to investigate the immune system in terms of healthy and unhealthy aging. Health status was defined by combining 34 health deficits into a frailty index. A subcohort (n=289, 60-85 yr.) was selected from DCS participants by random sampling, equally stratified by age, sex and health status. Absolute numbers of leukocyte subsets were characterized by multicolor flow cytometry. In addition, cytomegalovirus (CMV) serostatus and c-reactive protein (CRP) concentrations were measured. Our results revealed elevated numbers of neutrophils and monocytes in the frail population compared to the non-frail population. Furthermore, CRP trajectories (15 yr.) were higher in the frail population, consistent with inflammation and skewing of hematopoietic stem cells towards myeloid progenitors. Elevated memory (CD8) T cells were observed with age and CMV serostatus but not with frailty and could be part of a normal aging pathway. These differences in aging patterns of frail and non-frail populations make DCS subcohort promising for future research towards aging of the immune system. Identifying cellular immune markers and immune biomarker trajectories related to health status and age might help to predict future health problems and to explore intervention strategies to target immunological decline in the future.

P.A3.05.14

Analysis of alterations of HSP70 expression in peripheral immune cells of patients with obliterating arteriosclerosis

*A. Bornusova[1], E. Dzyubinskaya[2], **A. Sapozhnikov**[1,2];*
[1]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russian Federation, [2]Lomonosov Moscow State University, Moscow, Russian Federation.

In recent years, the role of humoral and cellular immunity in processes of atherosclerotic damages of vessels is being actively investigated. It was demonstrated that heat shock proteins HSP70 translocated on the surface of endothelial cells play an important role in development of autoimmune reactions peculiar to arteriosclerosis. It was also shown a considerable increase of extracellular pool of HSP70 and level of antibody to the protein in serum of peripheral blood obtained from patients with arteriosclerosis. In this work we investigated alterations of HSP70 expression in peripheral immune cells of patients with obliterating arteriosclerosis. The obtained results testify to significant differences between healthy donors and patients in a number of parameters connected with HSP70. In particular, our data demonstrated an increased content of inducible Hsp70 in lymphocytes isolated from peripheral blood of the patients as compared with healthy donors. Our preliminary results indicated also the presence of considerable level of surface HSP70 on lymphocytes obtained from a number of patients in contrast to healthy donor lymphocytes having no surface HSP70. Additionally our data confirm the results of other authors concerning an increased serum level of extracellular HSP70 and antibody to the protein in the blood of the patients. An essential addition to the literature data is connected with demonstrated in our study a significant positive correlation of the measured parameters mentioned above with the level of vessel calcification that reflects the development of arteriosclerosis. This work was partly supported by Russian Science Foundation, grant No. 16-15-10404.

P.A3.05.15

Analysing human peripheral B cell repertoire to predict rheumatoid arthritis

B. Szikora[1], *A. Marx*[1], *K. Kovács*[1], *O. Pipek*[2], *I. Csabai*[2], *I. Kacskovics*[1];
[1]*Department of Immunology, Eötvös Loránd University, Budapest, Hungary,* [2]*Department of Physics of Complex Systems, Eötvös Loránd University, Budapest, Hungary.*

B cells and the antibodies they produce are the most important elements of the humoral immune response. At the end of the B cell development, naïve B cells produce unique immunoglobulins, which are the basic elements of the immune repertoire. The diversity of these immunoglobulins (Ig) comes mainly from the various gene segments (V(D)J), randomly built-in nucleotides and somatic hypermutation during germinal center reaction. The healthy repertoire consists of many different Ig sequences with a relatively even distribution that can be altered due to infections or other disorders such as autoimmunity. In these diseases some B cells are activated, their numbers are increased and these clonal changes can be detected in peripheral blood. Current studies suggest that such alterations can be discovered and followed by analysing the Ig repertoire with the help of next-generation sequencing and bioinformatical evaluation. Rheumatoid arthritis (RA) is a common autoimmune disease, manifesting in synovial inflammation. Before the onset of RA, typical disease markers can be detected from blood: autoantibodies of given specificity, typically anti-citrullinated protein antibodies and/or IgM rheumatoid factors. However, these markers are not entirely reliable. Our aim is to establish a sensitive and accurate diagnostic marker that can detect highly expanded clones from the blood of RA patients to predict disease progression and follow treatment efficiency. Acknowledgments: Project no. FIEK_16-1-2016-0005 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the FIEK_16 funding scheme.

P.A3.05.16

Anti-atherosclerotic study of the macrophages polarization mediated by salidroside in atherosclerosis mice

X. Tong[1], *F. Huang*[2];
[1]*The First Affiliated Hospital, Kunming, China,* [2]*Yunnan University of Traditional Chinese Medicine, Kunming, China.*

The polarization of M1 and M2 macrophages has been proven to be closely related to Atherosclerosis (ATH). The purpose of this study was to investigate the role and potential mechanism of salidroside in the treatment of ATH mice.
ApoE-/- mice were fed with Western high-fat diet 12 weeks for the ATH models and devided into model group, positive group, salidroside group at random. Intragastric saline were given to model group. Atorvastatin (10mg/kg) was given to positive group. The salidroside group were administrated with salidroside of 100mg/kg daily for 8 weeks. The levels of serum lipids were checked and the aorta plaques were observed with Oil-red-O staining and Movat staining. Immunohistochemistry for F4/80, iNOS and CD206 were performed and the levels of cytokines MCP-1, IL-12p70, IL-6, L-1β, IL-10 and IL-13 were detected by ELISA.
The levels of TG, TC, and LDL-C in salidroside group were significantly lower ($P<0.01$) while the level of HDL-C was increased ($P<0.05$) when compared with model group. In histological changes less plaque areas were observed in positive group and salidroside group in comparison to model group. The expression of F4/80, iNOS, IL-6,IL-1β in positive group and salidroside group were significantly reduced ($P<0.05$) while the expression of CD206, IL-1β, IL-10, IL-13, IL-12p70 and MCP-1 were significantly increased when compared with model group ($P<0.01$).
Blood lipids might be regulated and ATh plaques might be stabilized by salidroside which might exert anti-inflammatory reactions by regulating the macrophages polarization.

P.A3.05.17

Immunological signatures of influenza vaccine responders and non-responders in the elderly

S. Trittel[1,2], *P. Riese*[1,2], *M. Akmatov*[3,2], *M. May*[4], *J. Prokein*[5], *T. Illig*[5,2], *C. Schindler*[4,2], *J. Huehn*[1,6], *F. Pessler*[3,2], *C. A. Guzmán*[1,2];
[1]*Helmholtz Centre for Infection Research, Braunschweig, Germany,* [2]*Centre for Individualized Infection Medicine (CiiM), Hannover, Germany,* [3]*TWINCORE, Hannover, Germany,* [4]*Clinical Research Center Hannover, Hannover Medical School, Hannover, Germany,* [5]*Hannover Unified Biobank, Hannover Medical School, Hannover, Germany,* [6]*Centre for Individualized Infection Medicine (CiiM), Braunschweig, Germany.*

Seasonal influenza outbreaks represent a serious public health problem leading to a significant economic burden and high mortality rates especially in risk groups, e.g. the elderly (≥ 65 years). Compared to younger vaccinees, the elderly show considerably reduced vaccine-induced protective immune responses. Although vaccines adapted to the needs of the elderly immune system are available (Fluad®), vaccine non-responsiveness is still a critical issue. The development of new or improved vaccination strategies and the identification of biomarkers predicting vaccine efficacy are thus urgently needed. Therefore, a clinical study was performed aimed at elucidating immunological mechanisms underlying influenza vaccine non-responsiveness in the elderly (n=234). Volunteers aged ≥ 65 years were vaccinated in the season 2014/15 or 2015/16 and blood samples (serum/plasma, PBMCs, whole blood) were collected before and at specific time points after vaccination. The stratification of the vaccinees by their increase in the hemagglutination inhibition titre against the three vaccine antigens allowed the identification of triple responders and triple non-responders. The immunophenotyping of PBMCs derived from these two distinct extreme groups revealed specific immunological signatures. Triple responders displayed enhanced functionality of T cell populations and NK cells whereas triple non-responders showed enhanced frequencies of suppressive regulatory T and B cells and reduced plasma levels of IL-8/IL-18 as compared to triple responders already before vaccination. These differences shed light on the mechanism underlying vaccine non-responsiveness and thus will contribute to the improvement of vaccination strategies. In addition, plasma cytokine levels might serve as putative biomarkers for the early stratification of vaccinees.

P.A3.05.18

Epigenetic changes in immune cells during ageing and inflammation

L. Tserel[1], *A. Salumets*[2], *K. Kisand*[1], *H. Peterson*[2], *K. Saks*[3], *R. Tamm*[3], *K. Kingo*[3], *A. Oras*[1], *R. Uibo*[1], *P. Peterson*[1];
[1]*Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia,* [2]*Institute of Computer Science, University of Tartu, Tartu, Estonia,* [3]*Tartu University Hospital, Tartu, Estonia.*

Ageing is a complex process occuring in every living organism. One of the most recognized consequences of ageing is a decline in immune function. Although the causes of ageing are still poorly understood, the link between DNA methylation and ageing has been very meticulously characterized in past decades. Changes in DNA methylation patterns occur gradually throughout an individual's lifespan and result in the age-related phenotypes. The accumulation of epigenetic modifications is part of normal ageing process. The aim of our study is to evaluate the changes in the numbers of different immune cells from whole blood using only site-specific DNA methylation analysis. We have chosen several distinctive CpG sites for different immune cell subpopulations and carried out sequencing to evaluate the methylation levels in young and elderly individuals. We correlated methylation values of specific CpG sites and cell numbers for different immune cells and created models for cellular quantification using only DNA methylation values. For example, we have created a model to calculate the number of $CD8^+$ TEMRA cells, that are considered to be the hallmark of ageing. The number of TEMRA cells increases in pro-inflammatory conditions of elderly and can therefore be used as a marker for inflammatory conditions. Our goal is to create cellular quantification models for different immune cell subpopulations and to use them as a potential biomarkers in different disease and health conditions.

P.A3.05.19

Human early pregnancy decidua is highly enriched for differentiated and proinflammatory gamma/delta T cells with diverse TCR repertoires

A. Terzieva[1], ***S. Zapryanova***[1], *I. Hristova*[2], *V. Dimitrova*[2], *L. Djerov*[2], *P. Dimitrova*[3], *T. Dimova*[1];
[1]*Institute of Biology and Immunology of Reproduction "acad. K. Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria,* [2]*Medical University, University Obstetrics and Gynecology Hospital "Mother House", Sofia, Bulgaria, Sofia, Bulgaria,* [3]*Institute of Microbiology "Acad. St. Angelov", Bulgarian Academy of Sciences, Sofia, Bulgaria.*

Introduction: During pregnancy the plasticity of maternal immune system is challenged by the requirement to tolerate a semi-allogeneic fetus while remaining vigilant against pathogen invasion. Lack of immune balance in the uterus can lead to excessive inflammation and pregnancy failure. Gamma/delta T cells are dual face fast-acting cells that bridge innate and adaptive immune system and have a role in the protection against infections, in tumor surveillance and in tissue repair in both inflammatory and metabolic stress. Our aim was to investigate the profile of maternal gamma/delta T cells during the establishment of successful pregnancy. Materials and methods: Decidual and blood samples from healthy pregnant women in early and term pregnancy and from non-pregnant women were examined by immunohistochemistry, flow cytometry and CDR3 size spectratyping to assess *in situ* distribution, differentiation, activation and migration potential of gamma/delta T cells as well as overall TCR repertoire diversity. Results: Our findings showed that 1) human early pregnancy decidua was enriched for activated, fully differentiated and pro-inflammatory γαμμα/δελτα T cells; 2) the expansion of gamma/delta T cells at materno-fetal interface was restricted to Vdelta1 subset; 3) CDR3delta's and CDR3gamma's repertoires were polyclonal except CDR3gamma9 which was oligoclonal regardless gamma/delta T cells location. Conclusions: Early pregnancy triggeres an influx of terminally differentiated and pro-inflammatory gamma/delta T cells with diverse TCR repertoires. **Acknowledgments:** This study is funded by Bulgarian National Science Fund, project DN 03/5.

P.A3.05.20

A peptide targeting inflammatory CNS lesions in the EAE rat model of multiple sclerosis

C. BOIZIAU;
INSERM U1026, Bordeaux, France.

Multiple sclerosis is characterized by inflammatory lesions dispersed throughout the central nervous system (CNS) leading to severe neurological handicap. Demyelination, axonal damage, and blood brain barrier alterations are hallmarks of this pathology, whose precise processes are not fully understood. In the experimental autoimmune encephalomyelitis (EAE) rat model that mimics many features of human multiple sclerosis, the phage display strategy was applied to select peptide ligands targeting inflammatory sites in CNS. Due to the large diversity of sequences after phage display selection, a bioinformatics procedure called PepTeam designed to identify peptides mimicking naturally occurring proteins was used, with the goal to predict peptides that were not background noise. We identified a circular peptide CLSTASNSC called Ph48 as an efficient binder of inflammatory regions of EAE CNS sections including small inflammatory lesions of both white and gray matter. Tested on human brain endothelial cells hCMEC/D3, Ph48 was able to bind efficiently when these cells were activated with IL1β to mimic inflammatory conditions. The peptide is therefore a candidate for further analyses of the molecular alterations in inflammatory lesions. This work was supported by grants from ANR, ARSEP, and Conseil Régional d'Aquitaine (France). KVS received a doctoral fellowship from the European Network Council ENC-Network.

P.A3.06 Immunomonitoring and biomarkers - Part 6

P.A3.06.01

Investigating the correlations between single nucleotide polymorphisms of immunity-related genes and cell surface markers of immune cells in porcine stocks in Taiwan

***A. Chen**, A. Liu, S. Liu, M. Wu, Y. Lien, H. Chaung;*
Research Center of Animal Biologics, Pingtung, Taiwan.

Single nucleotide polymorphism (SNP) is a genetic variation controlled gene expressions on transcriptional level. It has been reported that SNPs in immune-relevant genes influence the susceptibility of individuals to pathogens and diseases. The aims of this study are to investigate the distribution of SNP sites in immunity-related genes and its correlations to cell surface markers of immune cells within purebred and crossbred pigs in Taiwan. In this study, 39 SNPs of porcine immune-related genes (IFN-α, IFN-γ, TNF-α, GM-CSF, MCP-1, TLR3, TLR4, TLR7, TLR8 and TLR9) were identified and percentages of positive cells with cell surface markers of CD4, CD8, CD80/86, MHCI, and MHCII were analyzed from blood samples collected from 187 piglets at the age of 8 weeks (Landrace, Yorkshire, Landrace-Yorkshire hybrid, Duroc and Taiwan Black). The results showed that genotype distribution of 28 SNPs were significant associated with breeds, especially between Landrace and Taiwan Black. For instance, frequency of AG genotype of SNP-1 in Taiwan Black and Landrace were 1.6% and 13.4%, respectively. Among them, 18 SNPs significantly had impacts on the expressions of cell surface markers of CD4, CD8, CD80/86, MHCI and MHC II. Interestingly, CD4% (35.42±4.17) in AA genotype of SNP-28 was significantly higher than those in GG genotype (26.65±1.93) in all breeds. An understanding of these SNPs and their correlations with immunity parameters may be beneficial on establishing high pathogen-resistant breeding parameters. This genomic information aids in the discovery of SNPs in genes controlling disease resistance.

P.A3.06.02

Rapid and robust CD4+ and CD8+ T-, NK-, B-cell, and monocyte reconstitution after nicotinamide-expanded cord blood (NiCord) transplantation

***C. de Koning**[1], K. van Veghel[1], A. Lacna[1], L. Ebskamp-van Raaij[1], J. J. Boelens[2,1], S. Nierkens[1];*
[1]University Medical Centre Utrecht, Utrecht, Netherlands, [2]Wilhemina Children's Hospital, Utrecht, Netherlands.

Nicotinamide-expanded cord blood (NiCord) is a promising alternative source for allogeneic hematopoietic cell transplantation (HCT) when an appropriate donor is lacking.
We evaluated early immune reconstitution (IR) after NiCord HCT, in which especially CD4+ T-cell reconstitution is related for successful outcome.
In this phase1/2 multicenter trial, patients with hematologic malignancies received a NiCord-HCT after myeloablative conditioning. IR monitoring was performed in a random subgroup. Primary endpoint was CD4+-IR (>50*10 /L within 100 days). Secondary endpoints were IR of T-, natural killer (NK), B-cells, and monocytes during the first 6 months after HCT. Data were compared with cohorts of adolescent and young adult (AYA) patients at the UMC Utrecht receiving either unmanipulated cord blood transplantation (unCBT) or bone marrow transplantation (BMT) for hematological malignancy. Linear-mixed effects modelling in LOESS-regression curves and two-sided log-rank test for univariate comparisons in cumulative incidence plots were used.
36 NiCord recipients (median 41.5; 13.4-61.7yrs) were included, IR data was available from 22 patients. Of these patients, 91% achieved successful CD4+ IR; comparable to 27 unCBT (median 15.4; 12.2-22.1yrs) and 20 BMT (median 14.3; 12.1-19.7yrs) recipients (p=0.98). Overall T-cell IR was similar (p=0.53), while IR of NK-cells (p<0.001), B-cells (p=0.017) and monocytes (p<0.001), was faster after NiCord transplantation.
NiCord recipients had rapid and robust IR despite the younger age of the AYA cohorts receiving unCBT and BMT (expected to reconstitute faster). These data show the high proliferative capacity of the NiCord- expanded product in vivo, which will be further evaluated in an ongoing phase III multi-center trial.

P.A3.06.03

Identification of immune biomarkers in Tuberculosis patients and their contacts

***O. Estévez-Martínez**[1], E. Garet-Fernández[1], A. Martínez-Pérez[1], N. Fonseca[2], D. González-Peña[1], A. Pena[3], L. Barcia[3], Á. Pallares[4], L. Anibarro[3], Á. González-Fernández[1];*
[1]Cinbio (University of Vigo), Vigo, Spain, [2]European Bioinformatics Institute (EMBL-EBI), Cambridge, United Kingdom, [3]Tuberculosis Unit-Hospital Provincial Pontevedra, Pontevedra, Spain, [4]Microbiology Unit-Hospital Provincial Pontevedra, Pontevedra, Spain.

INTRODUCTION: The immunological mechanisms behind Tuberculosis (TB) infection or resistance are not yet fully characterized. A better understanding of these mechanisms could provide new biomarkers of the infection status. This would not only improve the diagnosis of new TB cases but also help on the identification of a different spectrum of contacts with latent infection. OBJECTIVE: Identification of a set of Tuberculosis biomarkers in blood, saliva and sputum samples. METHODOLOGY: We have recruited a Galician cohort of 28 newly diagnosed TB patients and 71 contacts exposed to the infection (44 uninfected -*NoTBI*- and 27 with latent infection -*LTBI*-). We have measured different cytokines and chemokines in serum, saliva and sputum by multiplex and studied the gene expression profile by RNAseq. Candidate biomarkers were selected based on the differential expression between groups (padj<0.05) and the fold change, and were used to create classification models. RESULTS: We have identified 6 protein markers in saliva and 4 in serum. Sputum did not allow us to select biomarkers. A set of genes differentiate TB patients from NoTBI contacts that partially overlap with a set of differentially expressed genes between TB patients and LTBI contacts. Genes differentiating NoTBI from TB were used to create a classification model that identifies different subsets of LTBI based on their similarity to infected patients. CONCLUSIONS: A group of biomarkers detected by different techniques characterizes the different status of TB infection and the different profiles of the latent infection.

P.A3.06.04

Anti-PD-1 Immunotherapy Modulates PD-L1 Expression on Neutrophil Subsets and Monocytes from Advanced Melanoma Patients

***M. R. Galdiero**[1,2,3], L. Cristinziano[1,2,3], M. Capone[4], G. Madonna[4], D. Mallardo[4], S. Loffredo[1,2,3], A. Ferrara[1,2,3], M. Braile[1,2,3], V. Vanella[4], L. Festino[4], P. Ascierto[4], G. Marone[1,2,3];*
[1]Department of Translational Medical Sciences (DiSMeT), Naples, Italy, [2]Center for Basic and Clinical Immunology Research (CISI), Naples, Italy, [3]WAO Center of Excellence, University of Naples Federico II, Naples, Italy, [4]Melanoma Cancer Immunotherapy and Innovative Therapy Unit, Istituto Nazionale Tumori Fondazione "G. Pascale, Naples, Italy.

Introduction: Advanced melanoma is a life-threatening cancer with a median survival of 6-9 months. Monoclonal antibodies (mAbs) that disrupt programmed death (PD-1) and PD-Ligand 1 (PD-L1) have revolutioned cancer immunotherapy. PD-L1 is expressed on several immune cells and recent evidence indicates that can be also expressed on human neutrophils. In addition to Normal Density Neutrophils (NDNs), a population of "Low Density" neutrophils (LDNs) increases in chronic inflammatory conditions and correlates with cancer progression. The role of peripheral blood neutrophils and monocytes as predictive biomarkers in anti-PD-1 therapy response is largely unknown. **Methods:** 39 Patients with advanced melanoma were prospectively recruited. PMNs and mononuclear cells were isolated from peripheral blood of healthy controls (HC) and melanoma patients, before and during anti-PD-1 therapy, to evaluate activation markers, PD-L1 expression, morphology, ROS production. **Results:** NDNs from melanoma patients displayed increased activation markers and PD-L1 levels compared to HC, which reverted during anti-PD-1 immunotherapy. Melanoma patients presented increased number of LDNs compared to HC but their percentages did not change during immunotherapy. Patients LDNs displayed increased PD-L1 expression compared to autologous NDNs which dropped after 3 months of therapy. PD-L1 expressing monocytes were increased in patients and decreased after 3 months of therapy. Patients NDNs showed reduced ROS production and peculiar morphological aspects. **Conclusions:** we found increased PD-L1 expression on peripheral blood NDNs, LDNs and monocytes in advanced melanoma patients, which was modulated by anti-PD-1 immunotherapy. Ongoing investigations are evaluating whether PD-L1 expressing myeloid cells can be associated with patient clinical outcome.

P.A3.06.05

Indoleamine 2,3 dioxygenase expression pattern in the tumor microenvironment predicts clinical outcome in early stage cervical cancer

***A. Heeren**, I. van Dijk, D. R. Berry, M. Khelil, D. Ferns, J. Kole, R. J. Musters, V. L. Thijssen, C. H. Mom, G. G. Kenter, M. C. Bleeker, T. D. de Gruijl, E. S. Jordanova;*
VUMC-CCA, Amsterdam, Netherlands.

Indoleamine 2,3-dioxygenase (IDO) can act as immunoregulator by inhibiting T cells via the degradation of tryptophan (trp) into kynurenine (kyn). The kyn/trp-ratio in serum is a prognostic factor for cervical cancer patients, however, information about the relationship between serum levels and IDO expression in the tumor microenvironment is lacking. IDO expression was studied in 71 cervical cancer samples by immunohistochemistry, and the link between kyn/trp-ratio in serum, clinicopathological characteristics, and the presence of T cells (CD8, Ki67, and FoxP3) in tumors were examined. In addition, we studied *IDO1* and *IFNG* gene expression using RNAseq data from 144 cervical tumor samples published by The Cancer Genome Atlas (TCGA). We demonstrate that patchy tumor IDO expression is associated with an increased systemic kyn/trp ratio in cervical cancer (P=0.009), whereas marginal tumor expression at the interface with the stroma is linked to improved disease-free and disease-specific survival (DFS: P=0.017, DSS: P=0.043). The latter may be related to T cell infiltration and localized IFNγ-release inducing IDO expression. Indeed, TCGA analysis revealed a positive correlation between *IDO1* and *IFNG* mRNA expression levels (P<0.001) and a significant association with improved DFS for high *IDO1* and *IFNG* levels. Our data indicate that the serum kyn/trp-ratio and IDO expression in primary tumors are not clear-cut biomarkers for prognosis and stratification of patients with cervical cancer for clinical trials implementing IDO inhibitors. Rather, a marginal IDO expression pattern in the tumor dominantly predicts favorable outcome, which appears to be related to IFNγ-release in the cervical tumor microenvironment.

P.A3.06.06

Observationally and genetically elevated plasma YKL-40 and risk of infectious disease in general population

***A. D. Kjaergaard**[1], J. Helby[2], J. S. Johansen[2], S. E. Bojesen[2], B. G. Nordestgaard[2];*
[1]Regionshospitalet Randers, Randers, Denmark, [2]Herlev and Gentofte University Hospital, Copenhagen, Denmark.

Background: YKL-40 is an acute phase protein elevated in patients with infectious and inflammatory diseases. We tested the hypothesis that baseline elevated YKL-40 is associated with increased risk of future infectious disease in the general population.
Methods: We performed prospective cohort and Mendelian randomization studies on 82,976 participants from the Danish general population followed for up to 23 years. We analyzed plasma YKL-40 levels (N=21,643) and *CHI3L1* (gene for YKL-40*)* rs4950928 genotype (N=82,375). Endpoints were any infection, bacterial pneumonia, diarrhoeal disease, sepsis, skin infection, urinary tract infection, and other infection.
Results: Multifactorially and CRP adjusted hazard ratio (HR) for any infection was 1.60 (95% CI: 1.40-1.83) for 91-100% versus 0-33% YKL-40 percentile category. Corresponding HRs were 1.80 (1.49-2.18) for bacterial pneumonia, 1.60 (1.08-2.37) for diarrhoeal disease, 1.63 (1.16-2.29) for sepsis, 1.64 (1.23-2.48) for skin infection, 1.54 (1.17-2.02) for urinary tract infection and 2.35 (1.18-4.70) for other infection. There was no difference between YKL-40 and CRP in the ability to predict risk of any infection (both area under the receiver operating characteristic (ROC) curve was 0.70). *CHI3L1* genotype was associated with plasma YKL-40 levels, but not with risk of any endpoint. Mendelian randomization did not support causality.
Conclusions: In the general population, elevated plasma YKL-40 levels were associated with increased risk of future infectious disease. The association was robust to extensive stratification as well as adjustment for confounders, including plasma CRP levels, but with no evidence to support causality.

P.A3.06.07

Impact of Radiofrequency ablation on plasma cytokines in patients with unresectable liver cancer

***K. Mazmishvili**[1], N. Kikodze[1], I. Pantsulaia[1], M. Iobadze[2], M. Mizandari[3], N. Janikashvili[1], T. Chikovani[1];*
[1]Department of Immunology, Tbilisi state medical university, Tbilisi, Georgia, [2]V.Bakhutashvili Institute of Medical Biotechnology, Tbilisi state medical university, Tbilisi, Georgia, [3]Department of Interventional Radiology, Tbilisi State Medical University, Tbilisi, Georgia.

Introduction: Radiofrequency ablation (RFA) is widely accepted interventional approach for liver cancer and has the advantages of high treatment efficacy and low complication risk. The various studies, including ours, have reported the immunomodulatory effects of RFA procedure on primary and metastatic liver cancer. The aim of this study was to explore the influence of RFA on the factors of tumor microenvironment including plasma cytokines.
Material and Methods: 10 patients aged 39 to 72 years (mean 55.1±11.2 years) with unresectable primary and metastatic hepatic tumors underwent RFA. Blood samples were collected from each patient and plasma cytokines (TGF-β, IL-10, IL-17, INFγ) were measured before and after 1 and 3 month of RFA treatment. Healthy age-matched volunteers were used for group comparison. The Mann-Whitney U test, Mc Nemar test and Wilcoxon rank test were applied for intergroup comparisons as appropriate.
Results: Serum IL-17, IL-10 and TGF-β and IL-17 levels were elevated in the patients with liver cancer compared to healthy volunteers. Decreased IL-10 and INF γ levels were reported after 1 and 3 month of RFA procedure, whilst there were not a significant changes in TGF-β and IL-17 levels after RFA treatment.
Conclusion. Changes in plasma cytokine levels in patients treated with RFA further edits the evidence on the immunomodulatory effects of RFA on tumor microenvironment.

P.A3.06.08

Analysis of the specific T cell repertoire against polyomavirus BKV

***A. Mohr**, A. Moosmann;*
DZIF Research Group "Host Control of Viral Latency and Reactivation" (HOCOVLAR), Helmholtz Center Munich, Munich, Germany.

BKV is a persistent virus, widespread in the population, and an important pathogen in immunocompromised persons. No specific treatment is currently available.
BKV-specific T cells are believed to be important mediators of antiviral protection, especially after transplantation. Previously, T cell epitopes of BKV antigens were identified, but mostly limited to a subset of BKV antigens and HLAs. Antiviral protection appears associated with certain HLAs, but candidates for protective epitopes have not been identified.
A more complete picture of BKV-specific T cell immunity covering all antigens and ranking epitopes for immunodominance would help understand immune protection and improve immunomonitoring and immunotherapy.
Therefore, we are studying the BKV-specific T cell response at the level of antigens, epitopes and HLA restriction in healthy donors. We use CD40-activated B cells loaded with peptide libraries to establish T cell clones and consecutively analyze function and phenotype of the T cells.
Our preliminary results indicate that individual BKV carriers recognize multiple epitopes per antigen. Analyses of ex vivo reactivity in ELISPOT (n=27) indicated that most donors recognize 2-5 antigens (out of 5). Specific T cell clones have been established, and their exact specificity and HLA restriction is being investigated.
We will proceed to determine the minimal epitopes and analyze the endogenous presentation and recognition of identified epitopes. The results of these studies will allow to identify the T cell repertoire at the level of antigen, epitope, and HLA in order to optimize monitoring and therapy of patients.

P.A3.06.09

Binding Immunoglobulin Protein as a potential target for immunotherapy during TB disease

***B. Motaung**, A. Loxton;*
Stellenbosch University, Cape Town, South Africa.

Background: Mycobacterium *tuberculosis* (M.*tb*) infection is one of the leading causes of mortality worldwide. Recent studies have highlighted the importance of BiP in cells, which can become a target in many diagnostic settings as it has been implicated in conditions including arthritis, cancer, bacterial infection and autoimmune diseases. In our studies, we are aiming to understand the expression differences of BiP in different M.*tb* infection stages to help us understand the change of function in immune cells in relation to infection stress. **Method:** Absolute BiP secretion levels were assessed in plasma samples using ELISA. This included participants at TB diagnosis (TBDx), TB Treatment group (Week 1, Month 2 and Month 6) and Healthy (unexposed) participants. BiP concentration results were analyzed using GraphPad Prism 7. **Results:** Secretion of BiP was comparable between newly diagnosed untreated pulmonary TB cases and healthy unexposed controls, with levels obtained in healthy group (42.64 µg/ml) and in TBDx (40.88 µg/ml). Highest levels of plasma BiP during treated TB was observed by W1 (mean 68.57 µg/ml) and declined by M2 with 60.92 µg/ml and M6 with 51.40 µg/ml. **Conclusion:** Detection of BiP in plasma samples indicated metabolic change in immune cells due to stress posed onto cells by M.*tb* burden. Even though not significant, we observed a decrease in the mean levels of BiP over the course of TB treatment which correlates with a reduction in the accumulation of unfolded polypeptides in the ER. This observation requires further testing in larger prospective studies.

P.A3.06.10

Association of the natural killer cells with the clinical complications in patients with common variable immunodeficiency: a new risk marker

***S. Padure**, M. Di Natale;*
Hospital General Universitario Gregorio Marañón, Madrid, Spain.

The common variable immunodeficiency (CVID) is a pimary immunodeficiency that consists of hypogammaglobulinemia IgG and IgM or IgA, associated to recurrent infeccions and poor response to vaccines. In this study we will evaluate the association between the NK cell levels and the complications developed in patients with CVID.Methods: A retrospective study of 50 patients with CVID. Quantifications of NK cells were made by flow cytometry of 4 colours with the phenotypic definition CD3-CD56 + / CD16 +. T de Student, Chi-cuadrado and logistic regression statistical tests were used to analyze the data. Results: Demographic variables - 23 men and 27 women. We observed levels significantly lower of NK cells in patients hospitalyzed with infections (75±83 vs 170±129 cells/uL, p = 0.03) especially pneumonia (104±90 vs 163±133 cells/uL, p = 0.19), also with lymphoproliferation (76±70 vs 163±130 cells/uL, p = 0.019) and autoimmunity (93±91 vs 162±130 cells/uL p = 0.16). We observed that 50% of patients with severe infections had NK levels < 50 cells/uL compared to only 15% of patients without this event (p = 0.017). The logistic regression analysis confirmed that patients with levels of NK cells < 50 cells/uL were more exposed at risk of developing severe infection or death. Conclusion: Based on the results, we can conclude that levels of NK cells < 50 cells/uL can be considered as a risk factor for poor evolution in patients with CVID. This could be useful in the process of selection of patients who need more frequent controls.

P.A3.06.11

The influence of lithium and valproic acid on proliferation and apoptosis of T lymphocytes in patients with bipolar disorder.

***K. Pietruczuk**[1], K. Lisowska[1], K. Grabowski[2], J. Landowski[2], J. Witkowski[1];*
[1]Department of Pathophysiology, Gdansk, Poland, [2]Clinic of Adult Psychiatry, Gdansk, Poland.

Bipolar disorder (BD) is a serious mental illness with cyclic alternations of mood. It is not only affecting patients' mood and behavior but also influences their immune system. Therefore, we evaluated the efficiency of the immune system of BD patients treated with two different mood stabilizers (lithium or valproic acid).
We investigated proliferation of T cells stimulated with anti-CD3 antibody using cytometric dividing cell tracking (DCT) method. We also assessed their susceptibility to apoptosis using staining with annexin-V and measuring intracellulary amount of protein Bax and Bcl-2. Expression of Bax and Bcl-2 was also analyzed with quantitative RT-PCR. Additionally, we investigated *in vitro* influence of lithium or valproic acid on these parameters of T lymphocytes.
T lymphocytes of BD patients, especially those treated with lithium, had reduced proliferation capacity compared to healthy people. *In vitro*, valproic acid in very high dose reduced the number of cell divisions and percentages of proliferating cells regardless of health status. Meanwhile lithium had no significant influence on proliferation parameters of patients' lymphocytes. Lymphocytes of BD patients were also more prone to apoptosis compared with healthy individuals, which was related to high expression of Bax. *In vitro*, both lithium and valproic acid protected patients' lymphocytes from apoptosis.
To conclude, these results showed that mood stabilizers used to prevent relapses of BD have strong anti-apoptotic effect on patients' T lymphocytes but they are not able to improve their proliferation.

P.A3.06.12

Deciphering the role of Macrophages in pituitary tumours development

***M. Principe**[1], M. Chanal[1], A. Vasiljevic[2], E. Jouanneau[3], A. Hennino[1], G. Raverot[1,4,5], P. Bertolino[1];*
[1]Centre de Recherche en Cancérologie de Lyon, Inserm U1052, CNRS UMR5286, Université Lyon 1, Lyon, France, [2]Centre de Pathologie Est, Groupment Hospitalier Est, Hospices Civils de Lyon, Bron, France, [3]Universite Lyon 1, Service de Neurochirurgie, Hopital Neurologique, Hospices Civils de Lyon, Bron, France, [4]Féderation d'Endocrinologie, Groupement Hospitalier Est, Hospices Civils de Lyon, Bron, France, [5]Faculté de Médecine Lyon Est, Université Lyon 1, Lyon, France.

INTRODUCTION: Pituitary tumours (PiTs)- the second intracranial neoplasm- present heterogenic characteristics based on hormonal expression and secretion. Even if most of the PiTs have slow progression, a subset of these exhibits an aggressive clinical course with recurrences despite surgery, radio- and chemotherapy. Our project aims at cartographing the immune landscape of PiT Microenvironment (PiTME) in order to identified new immune-therapeutic targets. METHODS: Cytofluorimetric and immunohistochemical (IHC) analysis on fresh resected human PiTs are used. *In vivo* transplantation of fresh human PiTs has been performed in in Rag2KO mice. RESULTS: Firstly, we report the presence of T, B, NK cells and Macrophages in PiTME in a cohort of 42 patients screened by cytofluometry. Gonadotroph tumours have a peculiar infiltration of $CD68^+$ Macrophages in comparison to Somatotroph tumors that instead show a T cells infiltrate (activated $CD44^+/CD8^+$ T cells). Secondly, we confirm our data on human tissue sections showing a significant increase in $CD163^+$ staining in Gonadotroph compared to Somatotroph tumors, and this positively correlates with local invasion, but not with the tumour size. Thirdly, *in vivo* injection of fresh human PiT shows a sustained recruitment of mouse Macrophages, suggesting their importance in supporting tumour growth. CONCLUSION: We identified different TME landscapes in PiTs (Somatotroph and Gonadotroph). Our work pinpoints the crucial role of CD68+/CD163+ macrophages in tumoral progression and invasion. Future experiments will aim to find out if the re-education of these Macrophages (e.g. anti-CSF1R) can affects the PiTME and slow down tumour growth.

P.A3.06.13

A longitudinal investigative study of dengue-specific B cell responses during natural infection.

***A. Rouers**, K. Kaur, Y. Toh, D. Sathiakumar, R. Appanna, G. Au, K. Fink;*
*Singapore Immunology Network, A*STAR, Singapore, Singapore, Singapore.*

Dengue is a viral disease for which the immune response can be both protective and detrimental. T and B cells are highly activated in patients with severe disease, and this massive activation inevitably affects the immune repertoire. T and B cells are also important for the generation of immune memory and subsequent protection against re-infection. However, this anti-dengue adaptive immune response is not well characterized. To this end, we deeply investigated the cellular and molecular phenotype of dengue-specific B cell responses in a longitudinal cohort of 68 individuals infected by dengue virus for the first time or the second time.
We identified plasmablasts and observed a dramatic increase of their frequency 1 week after fever and a diminution by the early convalescent phase (1month after fever). In addition, secondary infected subjects had higher percentages of plasmablasts than primary infected subjects. Interestingly, study of anti-dengue antibodies in the serum of patients showed that titers drop rapidly between 1 and 6 months after fever. By investigating the neutralization ability of these antibodies, we observed a peak of neutralization at 1 month after fever, which is even higher in secondary-infected subjects. B-cell Elispot assays have been performed to assess the long-lasting B cell memory responses against dengue and highlight a maintenance of anti-dengue memory B cell responses at least 6 months after fever.
This work, completed by deep sequencing of B cell populations and investigation of T cell responses, will lead to important insights in the field of dengue immunology and vaccinology.

P.A3.06.15

Influence of DCF on peripheralimmune responses in HPV+ Squamous Cell Carcinoma of the anus (SCCA)

***L. Spehner**[1], M. Jary[1,2], S. Kim[2], M. Kroemer[1,2], O. Adotevi[1,2], V. Westeel[1,2], C. Borg[1,2];*
[1]UMR1098/BFC/EFS, Besançon, France, [2]CHRU, Besançon, France.

Purpose: To investigate the influence of Docetaxel-Cisplatine and 5-Fluorouracil (DCF) systemic chemotherapy on the peripheral immune responses in HPV+ Squamous Cell carcinoma of the anus (SCCA). Experimental Design: The presence of T-cell responses after a short term T-cell assay (7 days) against HPV16 E6/E7 derived-peptivator and Telomerase derived-peptides was monitored in 17 healthy donors (HD) and 59 patients with SCCA using IFN-g ELISPOT assay, before (n=59) and after (n=51) DCF treatment. Expression of TIGIT and Foxp3 on T-cells collected from peripheral blood both in HD and SCCA-cancer patients was determined using flow cytometry. Results: A HPV16 E6/E7 specific T-cell repertoire was present in HD (2/17 and 1/17 respectively) and SCCA-cancer patients before (21/59 and 7/59 respectively) and after (17/51 and 10/51 respectively) DCF treatment. A Telomerase specific T-cell repertoire was present in HD (5/17) and patients before (21/59) and after (16/51) DCF treatment. TIGIT expression on CD4 T-cells was higher in patients (8.3% and 8.2% respectively before and after DCF treatment) compared to HD (4.8%) ($p<0.001$). Similarly, CD4+FoxP3+CD25+ T-cells were higher in patients before (5.2%) and after (5.1%) DCF treatment compared to HD ($p<0.05$). DCF treatment did not modify TIGIT and Foxp3 expression. Conclusion: These results confirmed the immunogenicity of HPV16 E6/E7 and Telomerase derived peptides in HPV+SCCA. Our results show for the first time that the intensity of HPV16 E6/E7 and Telomerase specific responses are increased in HPV+SCCA patients after DCF treatment. Expression of TIGIT and Foxp3 were higher in patients and were not modify by DCF treatment.

P.A3.06.16

Are CD163-macrophages related with BRAF-mutation and progression of papillary thyroid carcinoma?

O. Sulaieva[1], *O. Chernenko*[2], *O. Larin*[2];
[1]*Laboratory of Pathology CSD Health Care, Kyiv, Ukraine,* [2]*Ukrainian Research and Practical Centre for Endocrine Surgery, Kyiv, Ukraine.*

The aim of this study was to assess relationship between *BRAF*-mutation status and papillary thyroid carcinoma (PTC) immune microenvironment with focus on CD163 macrophages. 60 patients with histopathologically confirmed PTC (48 females and 12 males, 43.2±0,9 years old) were enrolled in the study. *BRAF* V600E mutation was detected by PCR-RT prior to surgery using fine needle aspiration biopsy. There were 28 patients with BRAF mutation (46.7%). Tumor size, histological type of PTC, extra thyroidal extension (ETE) and intrathyroid invasion (ITI) and regional lymph nodes metastases were considered. In addition, immunohistochemistry with antibodies against CD163, COX-2 and VEGF was performed. *BRAF* mutation was detected in 28 patients (46,7%) with PTC. We did not find a significant association between *BRAF* V600E mutation and PTC clinicopathological features such as tumor size, ETE, ITI and lymph node metastasis. However, it was the significant difference in count of tumor associated CD163 macrophages in patients with different BRAF-mutation status (P=0.02). Most of PTC with high CD163 cells number was conventional rather than follicular type. CD163 cells number tightly correlated with VEGF (r=0.825, p<0.0001) and COX-2 expression (r=0.798, P<0.0001) and was related with high microvascular density (P=0.006) and ITI features (P=0.03). High number of tumor infiltrating CD163 cells was also associated with increase of CD163+ cells number in lymph nodes (P=0.002) and PTC metastasis (OR 13.3; 95% CI 3.1-57.2; P=0.0005). Thus, count of tumor associated CD163-macrophages, rather than BRAF-mutation, is associated with PTC lymph node metastasis.

P.A3.06.17

Varicella Zoster Virus-IgG antibody avidity in dialysis and kidney transplant patients

L. Wang[1], *C. Rondaan*[2], *E. Eelsing*[1], *A. A. de Joode*[3], *J. Westra*[1], *N. A. Bos*[1];
[1]*Department of Rheumatology and Clinical Immunology, University Medical Center Groningen and University of Groningen, Groningen, Netherlands,* [2]*Department of Medical Microbiology, University Medical Center Groningen and University of Groningen, Groningen, Netherlands,* [3]*Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen and University of Groningen, Groningen, Netherlands.*

Introduction: Varicella zoster virus (VZV) infection usually occurs during childhood causing chickenpox establishing a lifelong latency, but can reactivate resulting in herpes zoster (HZ). Older persons and immunocompromised patients are at increased risk for developing HZ and related complications. The relation between functional affinity (avidity) of VZV-IgG and immunity against VZV reactivation has not been fully understood in immunocompromised patients. **Materials and Methods**: Serum samples and PBMCs were collected from 60 kidney transplant patients (KTx, median age: 55.6 years) approximately 3 years after transplantation, 63 patients on renal dialysis (RDs, 71.1 years) and 30 matched healthy controls (HC, 55.3 years). VZV-IgG level and relative avidity index (RAI) were assessed by glycoprotein (gp)VZV-ELISA with a denaturing agent (urea) incubation step. Cellular immunity (CMI) to VZV was measured by IFN-γ Elispot and expressed in spotforming units (SFU) per $5x10^5$ PBMCs. **Results**: There were no significant differences in IgG anti-VZV levels and RAI between the groups. CMI was higher in HC (128 SFU/$5x10^5$ PBMCs) compared to KTx (69 SFU/$5x10^5$ PBMCs, p=0.011) and RDs (82 SFU/$5x10^5$ PBMCs, p=0.094). In KTx, CMI correlated negatively to age (R^2=0.146, p=0.003). In KTx there was also a negative correlation between RAI and level of anti-VZV antibodies (R^2=0.189, p=0.0005) and age (R^2=0.088, p=0.021). No such correlations were found in HC and RDs. **Conclusion:** In kidney transplant patients both VZV- CMI and avidity of IgG anti-VZV antibodies decrease with age, while higher levels of VZV antibodies do not compensate for this. Especially older KTx are therefore at risk of HZ.

P.A3.06.18

Rotavirus antigen detection in children with gastroenteritis

N. Zotos[1], *P. Christodoulou*[1], *E. Tatsina*[2], *C. Mitsis*[1], *C. Briasoulis*[1], *K. Tolis*[1], *A. Zotou*[3], *G. Katagis*[1], *A. Pournou*[1];
[1]*General Hospital of Ioannina, Greece, Ioannina, Greece,* [2]*Papageorgiou Hospital, Thessaloniki, Greece,* [3]*University Hospital of Ioannina, Ioannina, Greece.*

Aim: The present study aims to record the frequency of detection of soluble Rotavirus antigen in stools of children with gastroenteritis. Method**:** During the 2016-2017 period, 198 stools of children with gastroenteritis were sent to the Microbiology Laboratory of our hospital. In the context of the laboratory investigation to identify the causative agent of the disease, feces were tested for the presence of a Rotavirus protein antigen by immunochromatographic method. Results: In a total of 198 diarrheal faeces tested, 82 (41.41%) were found positive for the presence of Rotavirus antigen, 116 (58.58%) negative Conclusions**:** Rotavirus immunochromatographic methods for detecting stools are methods that can be used to search for the virus and give a fairly reliable result. Rotaviruses are a very common cause of gastroenteritis in children. Their valid and timely detection in the stools can help in the immediate application of the appropriate treatment and avoid the unnecessary use of antibiotics.

P.A3.06.19

Rubella antibody incidence in children

N. Zotos[1], *E. Tatsina*[2], *L. Papageorgiou*[1], *M. Gianniki*[3], *P. Christodoulou*[1], *F. Adam*[1], *A. Zotou*[4], *E. Chrisostomou*[1], *A. Pournou*[1], *N. Tsifetaki*[1];
[1]*General Hospital of Ioannina, Greece, Ioannina, Greece,* [2]*Papageorgiou Hospital, Thessaloniki, Greece,* [3]*Agia Sofia Hospital, Athens, Greece,* [4]*University Hospital of Ioannina, Ioannina, Greece.*

Infection by Rubella Virus occurs mainly during childhood. However vaccination against Rubella Virus is part of the vaccination program. Rubella infection during pregnancy may result in the birth of a child suffering from Congenital Rubella Syndrome (CRS) Aim: To determine the incidence of antibodies against Rubella Virus in children during a two-year study Method: 394 serum samples from children (Greek and Immigrants) were tested for the presence of IgG and IgM antibodies against rubella virus from January 2015 until December 2017. They were 1 - 14 years old. ELISA (AxSYM , Abbott) was employed for the detection of the specific antibodies. Results: 394 children (48% boys and 52% girls) were tested.79% of the children tested were positive to IgG antibodies while 21% of the children were negative. Acute infection (positive IgM antibodies) was detected in 1% of the children. Immune deficiency was discovered more frequently in girls (57%) as well as in Greek children (67.5%) Conclusion: A high rate of IgG antibodies against Rubella Virus was detected. To note, more Greek girls were immune compromised. It is important to note that the high rate of immunity against rubella among immigrants is not attributed to the actual incidence of rubella but it reveals the fact that they have adapted successfully to Greek society. Moreover, a strong social approach is important, so as to increase the rate of immunized people, especially women of the reproductive age.

P.A3.06.20

Serological indication of acute infection by CMV and EBV in patients of a thirdgrade hospital

N. Zotos[1], *E. Tatsina*[2], *P. Christodoulou*[1], *M. Gerasimou*[1], *G. Katagis*[1], *N. Varsamis*[1], *A. Zotou*[3], *M. Gianniki*[4], *A. Pournou*[1], *N. Tsifetaki*[1];
[1]*General Hospital of Ioannina, Greece, Ioannina, Greece,* [2]*Papageorgiou Hospital, Ioannina, Greece,* [3]*University Hospital of Ioannina, Ioannina, Greece,* [4]*Agia Sofia Hospital, Athens, Greece.*

Aim: The aim of this study was to find positive IgM antibodies for CMV and EBV the simultaneously in patients with mononuclear syndrome. Material / Method: 2611 sera of patients for IgM antibodies against Epstein-Barr virus were examined. The tests were performed with ELISA. Also, 3558 patients' sera for IgM antibodies against CMV were tested by ELISA. Patients, whose sera were tested, were hospitalized in a hospital of Northwestern Greece during the last six years (2012-2017). Results: Of the 2611 sera tested for EBV, the following results were obtained: 128 (4.9%) positive, 75 (2.9%) marginal positive and 2408 (92.2%) negative for ANTI-VCA IgM. Of the 3558 sera tested for CMV, 94 (2.6%) positive, 35 (1%) marginal positive and 3429 (96.4%) negative for CMV IgM were obtained. All positive or marginal positive CMV IgM results were crossed with the corresponding results of EBV, in order to investigate the current IgM antibody positivity. All 129 positive sera for CMV IgM were tested for IgM against EBV. So it emerged that 37/129 (28.7%) had a simultaneous positivity. Of the 37 sera, 22 had high transaminases, leading to EBV infection, and positive IgM antibodies to CMV probably due to P62. Conclusions: Contemporary serological evidence of EBV and CMV co-infection has not been adequately explained whereas it is co-infection or endogenous reinfection. It appears, however, that EBV or CMV infection can lead to the synthesis of identical or approximately identical IgM antibodies.

P.A3.06.21

Interferon gamma transcript detection on T cells by combining three types of magnetic separation

S. Carinelli[1], *C. Xufre*[2], *M. Pividori*[1], ***M. Marti***[2];
[1]*Grup Sensors i Biosensors. Dpt Química. Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain,* [2]*Institut de Biotecnologia i Biomedicina. Universitat Autònoma de Barcelona, Bellaterra (Barcelona), Spain.*

Interferon-γ is a proinflammatory cytokine, and its production is related with effective host defense against intracellular pathogens. Therefore, the level of interferon-γ is considered a good biomarker for intracellular infections. Beside this, it is also useful for the assessment, treatment progression and follow-up of non-communicable diseases, including cancer and autoimmune disorders, among others. This work addresses the development of a new strategy to evaluate the expression of interferon-γ transcripts produced by stimulated T lymphocytes cells as biomarker. The method sequentially combined three different types of magnetic separation, including the immunomagnetic separation of the T lymphocytes performed with antiCD3 magnetic particles. After that, the isolation and preconcentration of polyadenylated mRNA followed by the multiplex double-tagging RT-PCR amplification of the interferon-γ and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) genes (as a housekeeping control) were performed on polydT magnetic particles. Finally, the multiplex electrochemical genosensing is performed on streptavidin magnetic particles as a support. This approach is able to quantify the levels of cellular interferon-γ produced by as low as 150 T cells with outstanding analytical features to be considered as a promising strategy for the quantification of this important biomarker for several clinical applications.

P.A3.07 Immunomonitoring and biomarkers - Part 7

P.A3.07.01
Folate deficiency exacerbates the elevation of inflammatory cytokines in a sodium fluoride-induced renal inflammatory murine model

***C. Chan**, B. Lin;*
Department of Biochemical Science and Technology, College of Life Science, National Taiwan University, Taipei, Taiwan.

Introduction: Inflammation, as well as nutritional status, is associated with chronic diseases. Folate deficiency impairs immune cells function and differentiation. Whether folate deficiency exaggerates inflammatory responses still need to be clarified. It has been shown that sodium fluoride (NaF) induced renal inflammatory responses. Therefore, in the present study, we investigate the effect of folate status on the NaF-induced renal inflammatory responses in mice.
Materials and Methods: Twelve-week-old C57BL/6 mice fed with AIN93 diet were randomly divided into four groups: without or with folic acid for 10 weeks, and further orally gavaged with NaF (48 mg/kg body weight, F0-NaF, F1-NaF), or PBS as the vehicle groups (F0-PBS, F1-PBS), respectively, for 4 weeks.
Results: Feed efficiency, initial and final body weight did not differ among groups, but lower food intake and serum folate under folate deficient (F0) diet. Although urine protein, urea and BUN did not differ among groups during 4 weeks' induction, the significantly highest serum creatinine level was found in the F0-NaF mice. Serum TGF-β1 level and inflammatory mediators IL-6, MCP-1, but not TNF-α, were significantly higher in folate-deficient mice, and further increased by NaF induction.
Conclusions: Folate deficiency and NaF are independent factors that enhanced inflammatory cytokines levels in mice.

P.A3.07.02
Isoelectrofocusing as a high-resolution tool for the characterization of monoclonal gammopathies

***J. I. Fernández**, P. E. Walo, A. Roncancio, E. Roldán, L. M. Villar, M. Espiño;*
Immunology Department, Hospital Universitario Ramón y Cajal, Madrid, Spain.

Introduction: Immunofixation electrophoresis (IFE) is considered the gold standard for characterization of monoclonal (M) proteins. However, IFE is not always easy to interpret. The presence of oligoclonal pattern or single diffuse M protein bands could generate confusion about the nature of M bands or their clonality. Isoelectric focusing followed by immunoblotting (IEF/IB) allows a greater analytical sensitivity and band resolution. We report a case that show the usefulness of this technique in charaterization of M components.
Materials and Methods: IFE was performed on the Hydrasys 2 Scan Focusing system using Sebia antisera. IEF were carried out following a method previously developed in our laboratory. In this procedure, ampholytes in the range pH 3–10 were used; proteins were transferred to PVDF membrane and revealed with anti-human IgM conjugated with alkaline phosphatase and anti-human kappa and lambda light chain antibodies conjugated with HRP.
Results: A 65-year-old male patient presented with anaemia, thalamic hemorrhage and IgM levels of 5.2 g/dl. Serum protein electrophoresis revealed a wide M speak in gamma region. A single monoclonal IgM band was characterized by IFE. This band was reactive with lambda and kappa light chain antisera. Pre-IFE reduction with DTT did not change double recognition of the IgM band by anti-kappa and lambda antibodies. However, IEF revealed the presence of two distinct separate bands: IgM kappa and IgM lambda indicating IgM biclonal gammopathy.
Conclusion: IEF/IB could be useful in the clinical setting to detect the clonality of M protein bands in problematic cases.

P.A3.07.03
Effect of chronic physical stress on immune system

***A. S. Hamada**, M. S. Shorbagy;*
Al Azhar university hospital, Cairo, Egypt.

Background: Physical stress affects most people in some way. During short-term stress, multiple physiological systems are activated to enable survival. Short-term stress response prepares the cardiovascular, musculoskeletal, and neuroendocrine and immune systems for fight-or-flight. On the contrary, stress can be harmful when it is chronic or long lasting as prolonged exposure to physical stress can negatively affect all body systems including immune system.
Objective: Investigating the effect of chronic physical stress on some parameters of immune system.
Patients and Methods: This study has been carried out on 50 truck drivers who work more than 72 hours per week, and 20 healthy persons of matched age and sex as a control group. Serum cortisol level (fasting, morning), IgG, IgM, IgA, C3 and C4 were measured, in addition to CBC and leucocyte differential count.
Results: Comparing results of patients group to normal control group, The patients group showed significant increase in cortisol(p=0.0024) , decrease in IgG and IgA levels (p=0.0122 and p=0.0089; respecively) and statistical significant increase in WBCs count in the case group(p=0.017). While C3,C4 and IgM levels showed insignificant change(p=0.0916, p=0.153 and p=0.111; respectively) .
Conclusions: Chronic physical stress suppresses the immune system and its function, subsequently, our study recommends adequate sleeping, healthy diet, reasonable exercise and to minimize physical stress as a life style to stay away from infections ,cancers and autoimmune disorders.

P.A3.07.04
T-Cell outcomes of HIV/AIDS treated patients with either EFAVIRENZ or NEVIRAPINE regimens in Yaoundé, Cameroon

***G. M. Ikomey**[1], B. Hycenta[1], G. Jacobs[2], M. Mesembe[1], E. Lyonga[1], A. Eyoh[1], M. Okomo Assoumou[1];*
[1]Virus Immunology Unit, Yaounde, Cameroon, [2]Division of Medical Virology, University of Stellenbosch, South Africa.

Introduction: T-cells responses provide information on immune failure or successes. Our study aimed to evaluate T-cell outcomes of patients on either Efavirenz (EFV) or Nevirapine (NVP) regimens after six months on therapy.
Method: A longitudinal study was conducted from May through December 2016. HIV-1 positive participants were enrolled. T-cell counts were done using standard methods.
Results: Of the 256 there were 108 (56%) females and 84 (44%) males, mean age of 39.3 ± 7.9 years. There were no significant increases in CD4 T-cell with p=0.3676 and 0.5662, respectively. Immunological failure rate of EFV and NVP were 37.0%, and 61.9% respectively. There was a significant change in CD4:CD8 with p=0.0444.
Conclusion: EFV regimen showed a better immunological failure compared to NVP. There was a significant change in CD4:CD8 ratio ranging from < 1 to >1.CD4/CD8 ratio could serve as a marker to monitor immune restitution of HIV/AIDS patients on ARV than CD4 absolute.

P.A3.07.05
Mechanismof Wenyangjianpi Prescription on The Treatment of Recurrent Spontaneous Abortion Caused by Maternal-FetalImmune Tolerance Based on Its Effects on The Balance of Th17/Treg Cells

***L. Jiang**;*
Gynecology, Kunming, China.

object:Recurrent Spontaneous Abortion(RSA) is a complicated disease in women of reproductive age, and its morbidity rate is on rising year by year.This article was explored Wenyangjianpi prescription possess a satisfactory effect in treating RSA may mediate by regulation of Th17/Treg cells balance. In this study, in vivo CBA/J×DBA/2 mice RSA model was established. The balance of Th17/Treg cells and their specific transcription factor and protein expressions are investigated. The theory that Wenyangjianpi prescription affects Th17/Treg cells balance to prevent RSA caused by maternal-fetal immune tolerance will be tested. Method: The CBA/J×BALB/C mice was established . Mice were divided into five groups including, Model group,control group and Wenyangjianpi prescription high, middle and lowdosage group.After 56 days which record Embryo absorption rate by flow cytometry. We tested Th17/Treg ratio in the peripheral blood each group. And also IL-6,CD130,p-STAT3,VEGF,VEGF Receptor,IL-27,IL-23 were tested with western blotting approach. Results: 1.Embryo absorption rate, Th17/ Treg cells ratio, CD130 and IL-23 of model group is higher than control group; the expression of IL-6,p-STAT3,VEGF, VEGF-Receptor, IL-27 of model group was lower than control group. 2. Th17/Treg ratio, CD130, and IL-23 of the three different dosage groups were lower than model group. IL-6,p-STAT3, VEGF, VEGFReceptor, IL-27 of the three different dosage groups were higher thanmodel group.
Conclusion: Wenyangjianpi prescription decreased Embryo loss rate through adjusting Th17/Treg cells balance.

P.A3.07.06
Comparison of mitogen induced proliferation levels of pediatric and adult healthy control groups by CFSE dilution assay

***U. C. Kucuksezer**[1], Z. Shoub F Elshari[1], I. Tahrali[1], S. Nepesov[2], G. Deniz[1], Y. Camcioglu[2];*
[1]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Dept. of Immunology, Istanbul, Turkey, [2]Istanbul University, Cerrahpaşa Faculty of Medicine, Dept. of Pediatrics, Istanbul, Turkey.

Introduction: Proliferation of antigen-responsive lymphocyte clones is the first-step in acquired immunity, which is important for development of effector functions. Tests investigating lymphocyte proliferation gains attendance both for immunology research and clinical diagnose. Proliferation tests requested from pediatric clinics for diagnose and follow-up of primary immune deficiency patients require healthy controls for comparison, however due to multiple reasons, it is hard to obtain blood samples from pediatric age groups. Concordance with adult healthy controls is a question.
This study aims to compare mitogen-induced proliferation values of pediatric and adult healthy control groups.

Materials and methods: Peripheral blood mononuclear cells (PBMC) isolated from freshly heparinized blood samples of pediatric (n=15) and adult (n=15) healthy control groups were stained with CFSE and stimulated with polyclonal activators; anti-CD2, -CD3 and -CD28 (CD-mix) and phytohemaglutinin (PHA). Proliferation levels of total lymphocytes were investigated on day +5 of cell culture. Independent samples T test was utilized for comparison of proliferation values of pediatric and adult healthy control groups.
Results: No comparable differences were observed for proliferation values of unstimulated (3.46±1.22% vs 3.70±2.12%), PHA stimulated (82.66±7.42% vs 75.59±9.12%) and CD-mix stimulated (82.96±7.15% vs 84.95±3.85%) proliferation values between pediatric and adult healthy control groups, respectively.
Discussion: The preliminary results of this study revealed similarity between pediatric and adult healthy controls. These findings may suggest the possible utilization of adult controls for comparison with pediatrics. More number of pediatric controls are needed, which will let the formation of specific pediatric age groups for more powerful comparisons.

P.A3.07.07
Mitogen-triggered proliferation responses of pediatric thalassemia major patients treated with bone marrow transplantation

U. C. Kucuksezer[1], *I. Tahrali*[1], *S. Nepesov*[2], *A. Yesilipek*[3], *G. Deniz*[1], *Y. Camcioglu*[2];
[1]*Istanbul University, Aziz Sancar Institute of Experimental Medicine, Dept. of Immunology, Istanbul, Turkey,* [2]*Istanbul University, Cerrahpaşa Faculty of Medicine, Dept. of Pediatrics, Istanbul, Turkey,* [3]*Bahcesehir University, Faculty of Medicine, Dept. of Pediatrics, Istanbul, Turkey.*

Introduction: Thalassemia major (TM), a disorder caused by gene mutations encoding globin chains of the hemoglobin molecule present with severe hemolytic anemia, impaired organs and have increased risk for infections due to deficient immune responses. The main way of permanent treatment for TM is bone marrow transplantation. Determination of cellular responses are required for vaccination timing after bone marrow transplantation. CFSE dilution is a flow cytometric approach for estimation of proliferative capacity.
Aim: This study aims to investigate mitogen-triggered proliferative responses of pediatric bone marrow transplant recipients with TM.
Methods: Peripheral blood mononuclear cells (PBMC) were isolated from freshly heparinized blood samples of TM patients after 1 year of bone marrow transplantation, and also from healthy subjects. PBMCs stained with CFSE were stimulated with polyclonal activators; anti-CD2, -CD3 and -CD28 (CD-mix) and phytohemaglutinin (PHA), and total, $CD4^+$ and $CD19^+$ lymphocyte proliferation levels were investigated on day +5 of cell culture.
Results: Cut-off values evaluated from healthy controls (n=20) were used for comparative evaluation of proliferative capacities of bone marrow recipients (n=23). 20 patients had normal proliferation values of total PBMCs, $CD4^+$ T cells and $CD19^+$ B cells, with no statistical significance in comparison with healthy controls. 3 patients were non-responsive to mitogens at various degrees.
Discussion: Our results reveal re-gained proliferative capacity in TM patients 1 year following bone marrow transplantation, which may be adequate for determination of vaccination time points. CFSE dilution can be a suitable tool for determining success of bone marrow transplant in immunological aspect.

P.A3.07.08
Identification of very early inflammatory markers in a porcine myocardial infarction model

E. López*, V. Álvarez, R. Blázquez, F. Marinaro, V. Crisóstomo, C. Báez, V. Blanco, A. Abad, J. García-Casado, F. Sánchez-Margallo;*
Centro de Cirugía de Mínima Invasión, Cáceres, Spain.

Introduction: The porcine model has been widely used to understand the pathophysiological mechanisms involved in cardiovascular disorders. The porcine myocardial infarction model is associated to an inflammatory response which includes elevation of circulating inflammatory cytokines, chemokines and activation of immune cells. This study aimed to identify which are the very early immune-related biomarkers that can be used as predictors of myocardial infarction severity.
Materials and Methods: Myocardial infarction model was created by 90 minutes temporary occlusion of the mid-left anterior descending artery. Peripheral blood samples were collected before and 1 hour after model creation. Peripheral blood leukocytes subpopulations were isolated by gradient density and analysed by flow cytometry. IL2, IFNγ, TNFα, IL12, IL10, IL4 and IL5 cytokines gene expression was analysed by RT-PCR.
Results: The analysis of lymphocyte subsets revealed a significant increase of CD4+/CD8+ ratio at 1 hour post-myocardial infarction. Regarding to the activation/differentiation status of T lymphocytes, the percentages of naïve, effector and effector/memory T cells did not show any significant change. Interestingly, CD4+ FoxP3+ cells were slightly increased after myocardial infarction. Concerning TH1/TH2 cytokines, a decrease in IL2, IFNγ, IL4 and IL5 gene expression was observed in peripheral blood cells.
Conclusions: The experimentally induced myocardial infarction modifies the distribution of T-cells and TH1/TH2 cytokines which is probably the consequence of lymphocyte recruitment to the inflammatory focus. Here we suggest that, the identification early biomarkers for myocardial infarction may provide very useful information for the diagnosis and treatment of this disease.

P.A3.07.09
Ubiquitin signalling in T cell activation: mapping the dynamic ubiquitylome

S. Matthews*, D. Kwasna, D. Pathak, Y. Kulathu;*
University of Dundee, Dundee, United Kingdom.

Precise control of T cell activation and differentiation is essential for competent adaptive immunity and deregulation of these processes can lead to lymphomas, autoimmunity and inflammation. The biochemical events regulating these processes have therefore long been a topic of intense research, which has been focussed predominantly on protein phosphorylation. It is now emerging that ubiquitin (Ub) and Ub-like protein (UBL) systems have important functions in regulating immune responses. The importance of ubiquitylation in adaptive immunity is underscored by the severe immunological disorders that arise when components of the ubiquitin system are disrupted in lymphocytes. However, we have a poor understanding of the pathways and mechanisms by which ubiquitin signalling regulates T cell function. This is largely due to the complexity of the ubiquitin system which makes it challenging to study this posttranslational modification. We here combine recently developed tools and quantitative proteomics to analyse dynamic changes in the Ub and UBL-modified proteome of activated T cells. Further, by analysing deubiquitinating enzymes, I will present our recent work that provides novel insights into the regulation of T cell function.

P.A3.07.10
TNF-Luc mouse: A Novel Model to Screen Anti-Inflammatory Drugs

F. MINSHAWI*, MIKE WHITE, WERNER MULLER, NEIL HUMPHREYS, DEAN JACKSON, ANTONY ADAMSON, AND STAMATIA PAPOUTSOPOULOU;*
University of Manchester, Manchester, United Kingdom.

In order to screen new potential anti-inflammatory drugs, it is vital to have an accurate and quantitative physiological model to measure inflammation and changes in inflammation. Tumor necrosis factor alpha (TNFalpha) is a major pro-inflammatory cytokine expressed at high levels in the immune response. We have generated a bacterial artificial chromosome that encompasses the human TNFalpha genomic region, and have engineered it to express luciferase under the control of natural human TNFalpha genomic sequences and generated a novel transgenic mouse line using this construct, we call the TNF-luc mouse.
We show that macrophages from TNF-luc mice have low resting levels of luminescence that is rapidly turned on in response to Lipopolysaccharide (LPS) stimulation *in vitro*. The luminescence response closely minics the production of soluble endogenous mouse TNF secretion indicating that the reporter gene expression from the human *Tnf* promoter in this transgenic mouse strain is similar to the regulation of the endogenous murine promoter. Several known NF-kb pathway inhibitors, but not a MAP pathway inhibitor, were able to suppress the luciferase expression. Moreover, Interleukin 10 (IL-10), a known anti-inflammatory cytokine inhibits luciferase expression in a dose dependent fashion. In conclusion, the TNF-Luc reporter mouse and cells derived from this mouse line is a valuable tool for screening of pro and anti-inflammatory compounds *in vitro*.

P.A3.07.11
NK CELLS SUBSETS IN MURINE CUTANEOUS MELANOMA EXPERIMENTAL MODEL

G. Isvoranu[1], *M. Surcel*[1,2], *R. Huica*[1,3], *A. Munteanu*[1], *I. Pirvu*[1], *D. Ciotaru*[1], *C. Ursaciuc*[1], ***M. Neagu***[1,2];
[1]*Victor Babes National Institute, Bucharest, Romania,* [2]*Faculty of Biology, University of Bucharest, Bucharest, Romania,* [3]*Carol Davila University of Pharmacy and Medicine, Bucharest, Romania.*

Recent findings on NK cell activation in cancer patients indicate that several important parameters, such as tumour capacity to modulate NK function and phenotype of NK cells require consideration for the choice of a NK-based therapy. In this study, we show in a melanoma-bearing mouse model that NK cells from the spleen are reduced as percentage and have different phenotypic characteristics than NK cells from the healthy controls. Methods. C57BL/6 mice, 8-10 weeks old were subjected to subcutaneous inoculation of B16F10 cells for developing cutaneous melanoma. Controls were sex and age-matched mice. After 21 days spleens were harvested and immediately assessed using flow cytometry analyses for NK cells populations (FACSCanto II flow cytometer with DIVA software). Results. Experimental data show a statistically significant reduction of the percentage of NK cells in melanoma-bearing mice compared to control animals. Analysis of NK cell subsets, defined by the differential expression CD27 and CD11b combination, indicated a significant difference in the distribution of NK cell subsets, namely the mature subset being dominant in control mice. Also, we found decreased CD43 and KLRG1 markers (commonly used for terminally mature NK cells), down-regulation of activating receptor NKp46 and up-regulation of inhibitory receptor Tim-3 in tumor-bearing mice. Our study has provided new insights into NK cell phenotype as future new approaches to cancer immunotherapy.Acknowledgement.This work was partially supported by Core Program, implemented with the support NASR, project no. 18.21.02.01.

P.A3.07.12

Virus-specific TH2 cells grow out following addition of IL-4 in cell culture

S. Pollastro[1], *M. De Bourayne*[2], *B. van Schaik*[1], *A. Jongejan*[1], *A. van Kampen*[1], *B. Maillère*[2], *N. de Vries*[1];
[1]*AMC, Amsterdam, Netherlands,* [2]*SIMOPRO, CEA, Saclay, France.*

Introduction: Virus-specific Th2 T cells show lower frequency and lower capacity to expand compared to their Th1 counterparts. This makes their identification and characterization challenging when using *in vitro* T cells assays. **Methods:** Here, using a quantitative sequencing-based T-cell receptor (TCR) fingerprinting of clones, we studied expansion of virus-specific T cell clones after *in vitro* stimulation with virus-derived peptides in different cytokine milieus (addition of IL-2 + IL-4, IL-2 alone or no cytokines). **Results:** Significantly higher clonal expansion occurred in IL-2 + IL-4 milieu compared to IL-2 alone, and in IL-2 alone compared to no cytokines. Specificity for the peptides was confirmed by sorting and sequencing of CD40L-expressing T-cells. Analysis of the expanded TCR clonotypes revealed that addition of IL-4 induces expansion of different TCR clones when compared to IL-2 alone or no cytokines. Finally, three-color fluorospot assay showed that increased production of IL-5 and IL-10 could be detected only in the presence of IL-4 + IL-2 milieu. **Conclusion:** Taken together these data validate sequencing-based TCR analysis of clonal proliferation, and indicate that addition of IL-4 is necessary to induced clonal expansion of antigen-specific T cell clones with a Th2 functional phenotype.

P.A3.07.13

CD73 surface expression is decreased during T cell activation - modulatory function of CD73 in inflammation

A. Rissiek[1], *E. Schneider*[1], *R. Winzer*[1], *I. Ricklefs*[1,2], *L. Glau*[1], *F. Raczkowski*[1], *C. E. Müller*[3], *F. Haag*[1], *H. Mittrücker*[1], *E. Tolosa*[1];
[1]*Institute of Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany,* [2]*Department of Pediatric Pulmonology and Allergy, University of Lübeck, Lübeck, Germany,* [3]*Pharmaceutical Institute, University of Bonn, Bonn, Germany.*

As a consequence of cell activation or death, adenosine triphosphate (ATP) is released from cells into the extracellular space, inducing autocrine and paracrine purinergic signaling and inflammation. The ectonucleotidases CD39 and CD73 sequentially hydrolyze pro-inflammatory ATP to anti-inflammatory adenosine (ADO) and therefore play a key role in the generation of an anti-inflammatory environment. Under steady-state conditions human T cells express low levels of CD39. Upon activation cell surface CD39 is upregulated and contributes to local ATP degradation. In contrast to CD39, CD73 is mostly expressed on naïve CD8 T cells, while effector and memory T cells are mostly devoid. Upon *in vitro* activation, CD8 T cells upregulated CD73 in the first 24 hours, followed by loss of CD73 expression on the cell surface. *NT5E* RNA increased during the first hours and returns to initial levels two days after activation. Notably, only the cells which were primordially $CD73^-$ or that lost CD73 upon activation upregulated activation markers and underwent proliferation. CD73 was not re-expressed up to three weeks after activation. This $CD39^+CD73^-$ phenotype was observed at sites of inflammation, *i.e.* in the synovial fluid of children with juvenile idiopathic arthritis, where a large fraction of CD8 T cells were $CD39^+$ and lacked CD73 expression. Interestingly, cell culture supernatants from *in vitro* activated T cells displayed CD73-specific AMPase activity, indicating that T cell-derived extracellular CD73 is functional and may contribute to the mitigation of the immune response in the context of acute and chronic inflammation.

P.A3.07.14

The effect of end stage renal disease of T lymphocyte subpopulations in pre and post dialysis patients

E. Sampani[1], *A. Fylaktou*[2], *M. Stangou*[1], *V. Nikolaidou*[2], *D. Asouchidou*[2], *D. Daikidou*[1], *A. Anastasiou*[2], *M. Chalkia*[3], *C. Dimitriadis*[1], *P. Giamalis*[1], *A. Papagianni*[1];
[1]*Department of Nephrology, Aristotle University of Thessaloniki, Thessaloniki, Greece,* [2]*Department of Immunology, National Peripheral Histocompatibility Center, Hippokration Hospital, Thessaloniki, Greece,* [3]*Department of Radiology, Hippokration Hospital, Thessaloniki, Greece.*

Introduction: End stage renal disease (ESRD) is associated with alterations in immune response The aim of this study was to assess changes in the T cell repertoire within ESRD patients on pre-and six-months post-dialysis state.
Methods: T cells subpopulations, namely CD3+CD4+, CD3+CD8+, Natural Killer cells (CD4+CD16+56+), Tregs (CD4+CD25+FoxP3+),CD8+CD28+,CD8+CD28- and CD4+CD28- cells, were isolated from whole blood samples using flow cytometry in 27 predialysis and 12 post-dialysis patients. The results were compared to 13 healthy controls.
Results: ESRD patients had reduced total lymphocyte number (1606±655μ/L vs. 2459±520μ/L, p<0.001). Furthermore the total numbersof CD4+ and CD8+ Tcells were significantly decreased (701.5±360.9μ/L vs. 1052.5±301.1μ/L, p=0.005) and (419.8±256μ/L vs. 478.8±194.7μ/L) respectively compared to healthy controls. Reduced total number of NK cells (238.3±141.2μ/L vs. 277.3±83.8μ/L) and Tregs (47.0±25.5μ/L vs. 58.9±24.4μ/L) was also noticed.
The frequencies of CD3+CD8+CD28+Tcells were decreased (48.5±22.1% vs. 58.2±17.0%), p=0.067), while the percentage of CD3+CD8+CD28- (55.2±21.9% vs. 37.4±15.4%p=0.022) was significantly increased. In 12 patients who had a follow up sample 6 months after renal replacement treatment, no differences were found except for Tregs subpopulation which decreased post-dialysis (5.0±2.9%vs. 7.5±1.3%, p=0.093). In 4/12 who commenced on peritoneal dialysis, CD3+CD4+CD28- subpopulations reduced (from 8.0±3.5% to 4.2±3.2%, p=0.068), in contrast to hemodialysis patients, who showed no difference.
Conclusions: Significant alterations within ESRD patients were noticed, with a reduction in CD4+, NK and Tregs and increased expression of CD8+CD28-cells. After dialysis a further reduction in Tregs happened while the changes in CD28 expression on CD4 T cells tended to return to normal in peritoneal dialysis.

P.A3.07.15

Immunophenotypic and clinical characterization of acute lymphoblastic leukemia at a public oncology reference center in Maranhão, Brazil

A. SERPA[1], *L. Pontes*[1], *E. Noronha*[2], *A. Azevedo-Santos*[1], *R. Oliveira*[3];
[1]*Federal University of Maranhão, São Luís, Brazil,* [2]*National Institute of Cancer, Rio de Janeiro, Brazil,* [3]*Centre of Clinical Research - HUUFMA, São Luís, Brazil.*

Introduction: Acutel ymphoid leukemias (ALL) are characterized by the exaggerated proliferation of precursor cells of the lymphoid lineage, caused by successive mutations in this maturative phase. ALL are immunophenotypically classified as B: B-I or pro-B, B-II or common LLAB, B-III or pre-B and B-IV or mature; and T-1 or pro-T, T-II or pre-T, TIII or cortical-T and T-IV or mature lineage ALL; and ALL with expression of one or two myeloid markers. Materials and Methods: the present study analyzed clinical and immunophenotypic data from 52 patients assisted by the State referral center. Results: stratification showed a higher frequency between 1 and 10 years (63,5%), males (53,8%) and subtype B (88,5%). In abhorrent phenotype analysis, CD33 expression was 31,8% (n = 7) in 22 patients diagnosed with ALL B, 33% (n = 1) in 3 patients diagnosed with ALL. CD13 positivity was evaluated in 34 patients with B-ALL testing positive in 29.4% (n = 10), in 5 patients with T-ALL had no positivity. Regarding the evolution of patients from the day of diagnosis to day 8 of the treatment, there wasn't a statistical difference in the variation of the global leukocyte count in diagnosis and on the eighth day of treatment in all subgroups of ALL. Conclusion: the study showed that the frequencies of ALL in the state of Maranhão are relatively similar to those seen in literature, they present abhorrent phenotypes and respond to the treatment used.

P.A3.07.16

Memory-like CD8+ T cells show contradictory functional properties under lymphopenic conditions

Y. Y. Silaeva[1], *A. A. Kalinina*[2], *L. M. Khromykh*[2], *D. B. Kazansky*[2];
[1]*Federal State Budget Institution of Sciences Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russian Federation,* [2]*Federal State Budgetary Institution "N.N. Blokhin Medical Research Center of Oncology" of the Ministry of Health of the Russian Federation, Moscow, Russian Federation.*

Introduction: One of the major surface markers that allow to define different populations of T cells are CD44 and CD62L. Expression of these markers on a surface of T-lymphocytes defines their migration pathways and functional status. It is known, that in lymphopenic conditions peripheral T cells undergo homeostatic proliferation and acquire the memory like surface phenotype $CD44^{hi}CD62L^{hi}$, but data about functional activity of these cells remain controversial. We studied relationship between surface expression of the markers CD44 and CD62L and functional properties of CD8+ T cells under lymphopenic conditions. Materials and Methods: First of all, we investigated allogenic immune response of memory cells in sublethally irradiated immunized and unimmunized mice. Secondly, we studied the allogeneic immune response of splenocytes of sublethally irradiated mice after adoptive transfer of syngeneic splenocytes from intact or immune mice. Results: In spite of the practically identical ratio of cells with the surface phenotype of naive and activated T-lymphocytes in spleens of intact and immune sublethally irradiated animals, the antigen-specific response of the memory cells was observed only in immunized animals. Although splenocytes of both intact and immunized mice after adoptive transfer to sublethally irradiated animals acquired the surface phenotype of activated T cells their primary and secondary immune responses in vitro and in vivo are significantly suppressed. Conclusion: Thus, we proved that surface expression of CD44 in not the only condition for T lymphocyte to acquire the functional properties of memory T-cell.

P.A3.07.17

Effect of the levels of vitamin D upon CD161 positives T cells in healthy Bulgarian women

E. S. Slavov[1], *K. G. Nancheva*[1], *V. N. Mateva*[1], *R. B. Lokova*[1], *A. V. Avramova*[2];
[1]*Trakia University, Stara Zagora, Bulgaria,* [2]*"Medical Center SELENA-L", Stara Zagora, Bulgaria.*

A pandemic of decreased vitamin D serum levels in humans could disrupt the immune tolerance via activation of autoreactive lymphocyte subpopulations. We studied the effect of vitamin D levels upon the distribution of CD4+CD161+, and CD8+CD161+ T-cells in healthy Bulgarian women. We also surveyed the effect of the working environment on these parameters. 62 women (age 47,7±9,6) were evaluated. They were divided in three groups, according to their working place: 23 industrial workers in shift work, 16 office workers, and 23 teachers. The percentage of CD4+CD161+ and CD8+CD161+ cells was analysed by flow cytometry. Total vitamin D level was evaluated by electrochemiluminescence.
We found that only 4,8% of the women had normal vitamin D levels (>30ng/ml), 22,2% had insufficiency (20-30ng/ml), 63,5% had deficiency (10-20ng/ml) and 9,5% had severe deficiency (<10ng/ml). A moderate inverse correlation (r=-0,3, p=0,037) between vitamin D levels and the percentage of CD8+CD161+ cells was calculated. We found no statistically significant differences in vitamin D levels, and the percentage of CD4+CD161+ T-lymphocytes according to the type of work. A statistically significant decreased percentage of CD8+CD161+ T-lymphocytes was found in the industrial workers group (4,7±2,6) compared to office workers (7,9±2,6 p=0,0001) and teachers (7,8±3,5 p=0,002).We may conclude that the majority of the cohort of Bulgarian women had vitamin D deficiency. Vitamin D exerts specific dose dependent regulation on CD4+161+ and CD8+CD161+ T-lymphocytes. Further studies are needed to clarify the impact of the epigenetic effects of working conditions on vitamin D mediated regulation of CD161 positives T-lymphocytes.

P.A3.07.18

Virus-associated subpopulations of T-lymphocytes forming antiviral immune response to tick-borne encephalitis vaccine

A. L. Sycheva*, M. V. Pogorelyy, E. A. Komech, I. V. Zvyagin, I. Z. Mamedov, Y. B. Lebedev;*
Shemyakin–Ovchinnikov Institute of bioorganic chemistry RAS, Moscow, Russian Federation.

For tick-borne encephalitis (TBE), as for many other viral diseases, the most promising approach to reduce morbidity and mortality is preventive immunization. Vaccination efficiency is evaluated by antibody titers as standard, whereas T-cells form antiviral response as well. Therefore, study of T-cell response to immunization could give new insight in antiviral defense and be useful for new vaccine developing.
For this purpose we took PBMC from 5 donors and isolated pairs of full, $CD4^+$, $CD8^+$, and $CD45RO^+$ leukocyte fractions at each of the six timepoints during immunization with TBE vaccine. At several timepoints small vaccine-associated populations (IFNγ or IL-2 producers and $CD38^+HLA\text{-}DR^{hi}$ T-cells) were collected. Total RNA was isolated, T-cell receptor (TCR) β-chain cDNA libraries were prepared and sequenced on Illumina HiSeq.
Deep TCR repertoire profiling was performed for all collected fractions. By implementing mathematical models we found hundreds of significantly expanded T-cell clonotypes. Many of these clonotypes was detected in repertoire of small vaccine-associated fractions. Single preferential peak of the expansion was observed for $CD4^+$ T-cell clonotypes, while $CD8^+$ clonotypes had several comparable peaks at different timepoints. The last evidence allows us hypothesize recruitment of distinct $CD8^+$ T-cell clones at various stages of immune response. Also in TCR repertoire of each donor we found clonotype clusters (with similar amino acid TCR sequence reflecting in presumably similar specificity) that had maximal concentration at the same timepoint. These new data will help deeper understand T-cell clonotypes behavior during immunization with inactivated vaccines.
The work was supported by RSF grant 15-15-00178.

P.A3.07.19

Using commercially available antibodies for flow cytometry of *Panthera* species lymphocytes: A pilot study

T. T. Sylvester*, S. D. Parsons, P. D. van Helden, M. A. Miller, A. G. Loxton;*
DST/NRF Centre of Excellence for Biomedical Tuberculosis Research/SAMRC Centre for TB Research, Cape Town, South Africa.

The immune response against tuberculosis in lions is still poorly defined and our understanding is hampered by the lack of lion specific reagents. The process for producing antibodies against a specific antigen is laborious and not available to many research laboratories. As the search for antibody cross-reactivity is an important strategy for immunological studies in veterinary medicine, we have investigated the use of commercially available antibodies to characterize T cell subsets in African lions (*Panthera leo)*. We screened commercially available antibodies and investigated the influence of two different sample processing methods, as well as the effect of time delay on cell surface marker expression on lion lymphocytes. Using commercially available antibodies, we were able to identify CD4+, CD5+, CD8+, CD14+, CD25+, CD44+ and CD45+ T lymphocytes in samples obtained by density gradient centrifugation as well as red cell lysis of lion whole blood. Two distinct lymphocyte populations, which differed in size and phenotype, were observed in the samples processed by density gradient centrifugation. In this study we demonstrate the utility of commercially available antibodies to differentiate between T lymphocyte subsets including immune effector cells in African lion whole blood.

P.A3.07.20

Streptococcus pneumoniae antigen detection in urines of pneumoniae patients

N. Zotos[1], *P. Christodoulou*[1], *E. Tatsina*[2], *L. Papageorgiou*[1], *E. Chrisostomou*[1], *G. Katagis*[1], *A. Zotou*[1], *N. Varsamis*[1], *M. Gianniki*[3], *A. Pournou*[1], *N. Tsifetaki*[1];
[1]*General Hospital of Ioannina, Greece, Ioannina, Greece,* [2]*Papageorgiou Hospital, Thessaloniki, Greece,* [3]*Agia Sofia Hospital, Athens, Greece.*

Aim: The goal of this study is to record the frequency of detection of soluble pneumococcal antigen in human urine. Material and Methods: During the years 2014-2017, 505 samples of patients' (treated with pneumonia symptoms) urine were examined at the hospital's Microbiology Laboratory. Urine was tested for pneumococcal antigen detection by immunochromatographic method. The method detects the 23 serological types of streptococcal pneumonia that cause at least 90% of serious pneumococcal infections worldwide. With this method, it is possible to detect pneumococcal antigens when they are secreted at concentrations $>10^5$ cells / ml of urine. Results: A total of 505 urine specimens tested, positive for pneumococcal antigen present were 89 (17.62%), while 416 (82.37%) were negative. Conclusions: The immunochromatographic method of detecting urinary pneumococcal antigen is a fairly reliable laboratory method that contributes to the immediate identification of the pneumococcal agent. However, both a negative and a positive result should always be confirmed by sputum or bronchial secretion cultures.

P.A4.01 Germinal centers and B cell differentiation - Part 1

P.A4.01.01

Distinct subsets of isotype-switched memory B cells in murine bone marrow and spleen

R. K. Addo[1], *R. Riedel*[1,2], *P. Durek*[1], *J. Kummer*[1], *G. A. Heinz*[1], *V. Greiff*[1], *D. Schulz*[1], *C. Klaeden*[1], *F. Heinrich*[1], *U. Menzel*[2], *S. Kröger*[3], *U. Stervbo*[1], *R. Köhler*[1], *C. Haftmann*[4], *S. Kühnel*[1], *K. Lehmann*[1], *P. Maschmeyer*[1], *M. McGrath*[1], *S. Naundorf*[1], *S. Hahne*[1], *S. T. Reddy*[2], *A. E. Hauser*[1], *M. Mashreghi*[1], *H. Chang*[1], *A. Radbruch*[1];
[1]*Deutsches Rheuma-Forschungszentrum, Berlin, Germany,* [2]*Eidgenössische Technische Hochschule (ETH Zürich), Basel, Switzerland,* [3]*Humboldt-Universität zu Berlin, Berlin, Germany,* [4]*Universitätsspital Zürich, Switzerland, Zürich, Switzerland.*

It is known that bone marrow contains resident memory T lymphocytes and longlived memory plasma cells, in niches organized by mesenchymal stromal cells. Here we demonstrate that bone marrow also maintains a distinct population of isotype-switched memory B lymphocytes. These cells differ from memory B lymphocytes of the spleen expressing the same isotype, in terms of phenotype and B cell receptor repertoire. Switched memory B cells from spleen and bone marrow show a significant fraction of exclusive B cell receptors. While all cells of the spleen express CD62L and CD21, only about 50% of switched memory B cells of bone marrow express CD62L, and 50% have downregulated CD21, pointing to functional differences in recall responses. More than 90% of the switched memory B lymphocytes of bone marrow and spleen rest in terms of proliferation, according to staining with Ki-67 and resistance to Cyclophosphamide treatment. In the bone marrow, switched memory B cells localize individually to VCAM-1+ and fibronectin+ stromal cells, similar to bone marrow-resident memory T and plasma cells. Like those, resident and resting switched memory B cells of the bone marrow, as a distinct compartment of B cell memory, probably provide longterm memory to systemic antigens.

P.A4.01.02
Toll-like receptor 9 signaling antagonizes cognate B cell-helper T cell interactions

***M. Akkaya**, B. Akkaya, A. S. Kim, P. Miozzo, H. Sohn, S. K. Pierce;*
National Institutes of Health, Rockville, United States.

Ability of B cells to capture antigens in order to process and present to follicular helper T cells plays an important role in T cell dependent antibody responses such as germinal center formation and affinity maturation. However, our understanding of the regulation of these key events is incomplete. Here we show that stimulation of B cells through TLR9, while enhancing cytokine production, proliferation and IgM secretion, blocked the ability of B cells to capture, process and present antigens. In the presence of TLR9 agonist CpG, B cells were less able to pull antigens from membranes, and to deliver captured antigens to MHC loading compartments which in turn resulted in less peptide MHC complexes on cell surface. This decreased the duration of B-T interaction and caused a less efficient activation of antigen specific T cells. RNA seq experiment of B cells stimulated through BCR and/or TLR9 generated a non-overlapping principle component analysis indicating that TLR9 dependent inhibition of BCR mediated activities are through a novel transcriptomal program. Using a chimeric mouse model and serum samples from a human clinical trial, we showed that CpG, enhanced the magnitude of the antibody response to a protein vaccine but failed to promote affinity maturation. Thus, TLR9 signaling may enhance the level of antibody response at the expense of the ability of B cells to engage in germinal center events that are highly dependent on antigen capture and presentation.

P.A4.01.03
Antigen stimulated B cells require a second signal to maintain their initial metabolic boost and avoid activation induced mitochondrial dysfunction.

***M. Akkaya**, J. Traba, A. S. Roesler, P. Miozzo, B. Akkaya, H. Sohn, B. P. Theall, M. N. Sack, S. K. Pierce;*
National Institutes of Health, Rockville, United States.

Events that follow B cell activation and lead to proliferation and differentiation into antibody secreting cells are tightly controlled in order to prevent unwanted immune responses. Requirement for two temporally distinct stimuli, first provided by BCR stimulation and second by either T helper cells or TLRs is the key to this control mechanism. However, our knowledge on the interplay between these two signals is incomplete. Here, using in vitro and in vivo models, we showed that antigen binding to BCR rapidly increases both oxidative phosphorylation and glycolysis, however most of the early events following BCR stimulation such as spreading and contraction of antigen bound BCR and internalization of the bound antigen are performed entirely by using the energy produced by oxidative phosphorylation. Changes in gene expression and cellular remodeling resulting in increased glucose uptake and mitochondrial performance follow the initial activation, preparing the cells to respond to a second signal. However, if the second signal is not received in a timely manner, B cells undergo progressive loss of mitochondria function ultimately leading to apoptosis. Mitochondria dysfunction is a result of the gradual accumulation of intracellular calcium which leads to inefficient oxidative phosphorylation and increased ROS production and eventually swollen patches of mitochondria. Receiving a secondary signal within 9 hours of initial BCR signaling can prevent these pathological changes. Thus, antigen binding activates a metabolic program that imposes a short time window in which B cells either receive a second signal and survive or alternatively face elimination.

P.A4.01.04
Type I interferon remodels the lung to promote ectopic GC formation during influenza A virus infection

***A. E. Denton**[1], S. Innocentin[1], E. J. Carr[1], B. Bradford[2], F. Lafouresse[3], M. A. Neil[2], B. Ludewig[4], J. R. Groom[3], K. L. Good-Jacobson[5], M. A. Linterman[1];*
[1]Lymphocyte Signalling and Development, Babraham Institute, Cambridge, United Kingdom, [2]The Roslin Institute and the Royal (Dick) School of Veterinary Sciences, University of Edinburgh, Edinburgh, United Kingdom, [3]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [4]Institute of Immunobiology,, St Gallen, Switzerland, [5]Infection and Immunity Program, Biomedicine Discovery Institute, Monash University, Melbourne, Australia.

Ectopic lymphoid structures form in a wide range of inflammatory conditions, including infection, autoimmune disease and cancer. Despite their relative ubiquity, little is known about the mechanism(s) through which inflammation is able to convert a peripheral tissue into one that resembles a secondary lymphoid organ. Here we show that type I interferon produced after pulmonary viral infection induces CXCL13 in lung fibroblasts, driving CXCR5-dependent recruitment of B cells and initiating ectopic germinal centre formation. This identifies type I IFN signalling as a novel inducer of CXCL13 and demonstrates that this cytokine can promote lung remodelling, converting a non-lymphoid tissue to one capable of supporting a highly ordered functional tertiary lymphoid structure.
Biotechnology and Biological Sciences Research Council grant support: BBS/E/B/000C040, BBS/E/B/000C0427, BB/N011740/1

P.A4.01.05
Unfolded protein response triggers metalloprotease-mediated processing of BAFFR and TACI in B cells

*C. R. Smulski[1], P. Odermatt[1], L. M. Seidel[1], S. Herr[1], P. Schneider[2], **H. Eibel**[1];*
[1]University Medical Center Freiburg, Freiburg, Germany, [2]University of Lausanne, Lausanne, Switzerland.

BAFF-receptor and TACI are members of the TNF-receptor superfamily. They are expressed specifically by B cells. Binding of BAFF as a common ligand for both receptors initiates pro-survival signals by BAFFR and class-switch recombination by TACI. Processing of BAFFR and TACI modulates the survival of resting and of germinal center B cells as well as the levels of circulating BAFF. We recently have shown that the membrane metalloproteases ADAM10 and ADAM17 can both process BAFFR and TACI at the plasma membrane and that the proteolytic cleavage of both receptors is regulated by the degree of BAFF oligomerization. Now we report that different signaling pathways can activate selectively the processing of BAFFR and TACI by ADAM10 and by ADAM17 in a ligand-independent manner. Inhibition of proteasomal activity by compounds like bortezomib induces the unfolded protein response, generates reactive oxygen species (ROS) and activates the TRAF2-ASK1 pathway. The newly produced ROS then selectively enhance the constitutive processing of TACI by ADAM10, whereas ASK1 induces processing of BAFFR by ADAM17. This unexpected role of the unfolded protein response and of ROS production affects the ability of primary human B cells to survive in response to BAFF but not to CD40L. Our results provide new insight into the treatment of autoimmunity and multiple myeloma

P.A4.01.06
A molecular signature of human regulatory B cells

***A. Grasseau**, M. Boudigou, Q. Simon, D. Cornec, J. Pers, S. Hillion;*
INSERM UMR 1227, Brest, France.

Regulatory B cell (Breg) in human is a large group of B cell subpopulations with a large heterogeneity in phenotypes and suppressor mechanisms. This variability leads to a high difficulty to characterize Bregs. It is now possible to define a molecular signature identifying a cell function using high throughput sequencing approach. One aim of our work is to better characterize a molecular signature of Breg in order to better understand their characteristics and functions in human physiology.Our laboratory has developed an in vitro model to polarize B cells in Breg and effector B cell. We have performed RNA-sequencing on these opposed polarized cell and underlined 225 significantly differentially expressed genes (DEG) in Breg related to effector B cells.A “DAVID” analysis shows that upregulated DEGs are linked to cytokine production and immune regulation, such as IL-10 . PCA analysis performed on co-culture supernatants confirms the important modification of cytokine production between the two conditions. Moreover, downregulated DEGs are related to the interferon type-I pathway. Furthermore, several genes from DEGs are related to plasmablast/plasma cell differentiation pathways, such as PRDM1. We thus have confirmed that Breg function is dependent of a unique differentiation process through a “GSEA” analysis using public data. In conclusion, we demonstrated that B-cell functions could be modulated by a specific microenvironment and cellular interactions. Overall, Bregs could be seen as a continuum of various phenotypes that associates distinct cell surface markers, cytokine secretion and transcriptional regulator expression driving by a specific differentiation program.

P.A4.01.07
Characterization of distinct functional domains of Pax5 *in vivo*

***S. Gruenbacher**, L. Hill, D. Kostanova Poliakova, M. Busslinger;*
Research Institute of Molecular Pathology, Vienna, Austria.

B lymphocytes express a vast diversity of antigen receptors on their cell surface, which provide immunity against foreign pathogens. During B cell development, the transcription factor Pax5 tailors the gene expression profile in favor of B cells by both activating B cell-specific genes and inhibiting B-lineage inappropriate genes. By doing so, Pax5 induces B cell commitment at the pro-B cell stage and controls the maintenance of B cell identity throughout B cell development. Consistent with this, B-lymphopoiesis is arrested at an early uncommitted progenitor stage in the absence of Pax5.
The Pax5 protein consists of different evolutionarily conserved domains, some of which have been mutated by amino acid substitutions or truncations in B-cell acute lymphoblastic leukemia. For instance, the introduction of a premature stop codon has eliminated the C-terminal transactivation domain and adjacent inhibitory region, suggesting that these domains are crucial for the function of Pax5. So far, the different domains have been functionally characterized only *in vitro* in cell lines. To elucidate the function of the different Pax5 domains *in vivo*, we generated several *Pax5* mutant mice lacking individual domains. Interestingly, mice harboring a C-terminal truncation of Pax5 showed a developmental arrest at the onset of B-lymphopoiesis, similar to what has been observed in *Pax5* knockout mice. However, another mutant containing the first few amino acids of the transactivation domain could facilitate transcriptional activation of selected Pax5 target genes, while B cell development was still arrested at an early pro-B cell-like stage.

P.A4.01.08

Early emergence of the B cell lineage in developing zebrafish

***P. GUGLIELMI**, N. Abdellaoui, K. Kissa, G. Lutfalla, M. Nguyen Chi;*
DIMNP, UMR5235, Montpellier, France.

Our IGBP1 fluorescent transgenic zebrafish line identifies B lymphocytes throughout all maturation stages. B lymphocyte precursors are present on the yolk sac starting 2.5 dpf. Subsequently B lineage cells populate kidney, skin and gut. Use of double transgenic fish with IGBP1- and either *runx1*-, *kdr*- or *itga2b* (CD41)- reporters delineates two types of early B lymphocytes in embryos and larvae: 1/ a B cell population bearing the hallmarks of HSC progeny (*runx1*$^+$, *kdr*$^+$ and *itga2b*$^+$); 2/ an independent B cell lineage originating from yolk sac derived progenitors. After inhibition of HSC formation with a *runx1* morpholino, we still observed the yolk sack progenitor lineage. Functional transcripts for μ and ζ heavy chains were first detected in 11-12 dpf larvae with a limited repertoire. At 19 dpf and subsequently, H chain mRNAs pertaining to all VH subgroups were consistently present. Functional L chain transcripts were only found after 30 dpf. However, a germline transcription of the chromosome 19 L chain locus occurred starting 7 dpf. The resulting transcripts were processed using several cryptic splice sites present within the VL exons and upstream of the JL exon. Taken together, these results point to the existence of two separate developmental pathways for zebrafish B lymphocytes that could be reminiscent of the B1 and B2 type of differentiation in mammals. In addition, the existence of B cell precursors without Ig chain expression and the asynchronous appearance of H and L chain transcripts suggest respectively the existence of pro-B and pre-B cell maturation stages.

P.A4.01.09

The KDM4A/KDM4C/NF-κB and WDR5 epigenetic cascade regulates the activation of B cells

***K. Hung**[1], Y. Woo[2], I. Lin[1], C. Liu[1,3], L. Wang[4], H. Chen[1], B. Chiang[4,5], K. Lin[1];*
[1]Genomics Research Center, Academia Sinica, Taipei, Taiwan, [2]Division of Biological Sciences, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia, [3]PhD Program in Translational Medicine, Kaohsiung Medical University and Academia Sinica, Division of Allergy, Immunology and Rheumatology, Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, New Taipei City, Taiwan, [4]Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan, [5]Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan.

How epigenetic regulation participates in the function of transcription factors during the activation and differentiation of B cells is largely unknown. Here, we demonstrated that the histone demethylases KDM4A and KDM4C are up-regulated in stimulated mouse splenic B cells, concomitantly linked with the global down-regulation of histone modification of their substrates H3K9me3/me2. KDM4A and KDM4C are important for the coordinated regulation of activation and proliferation of stimulated B cells. Upon chromatin immunoprecipitation sequencing (ChIP-Seq) and *de novo* motif analysis, we identified NF-κB p65 as the binding partner of KDM4A and KDM4C. Their co-targeting to *Wdr5*, a MLL complex member promoting H3K4 methylation, results in the up-regulation of cell cycle inhibitors, including *Cdkn2c* and *Cdkn3*. Thus, B cell activation is accompanied by KDM4A and KDM4C induction that initiates a stepwise epigenetic cascade for the proper control of the proliferation of activated B cells. We also assess whether the regulation of KDM4A/KDM4C/NF-κB/WDR5-mediated CDKN2C and CDKN3 gene regulatory cascades is disturbed in stimulated B cells of systemic lupus erythematosus (SLE) patients and found that the up-regulation of KDM4A and KDM4C as well as the induction of WDR5 are impaired in stimulated SLE B cells, as compared those in stimulated B cells from healthy donors. Together, in this study, we uncovered a stepwise epigenetic regulatory cascade that regulates B cell proliferation and activation.

P.A4.01.10

B cell-intrinsic role of DOCK2 inT cell-dependent humoral immunity

***Y. Kamikaseda**[1], M. Ushijima[1], Y. Fukui[1,2];*
[1]Medical Institute of Bioregulation Kyushu University, fukuoka city, Japan, [2], Reseach Center for Advanced Immunology, Kyushu University, Fukuoka city, Japan.

Introduction: Although activation of the small GTPase Rac has been implicated in BCR-mediated antigen recognition, its precise role in humoral immunity and the upstream regulator remain elusive. In this study, we examined B cell-intrinsic roleof DOCK2 in plasma cell (PC) differentiation and antibody production.Material and methods: We took the following three approaches: 1) B cells from conventional DOCK2 KO mice and control mice were used for biochemical analyses and in vitro functional assays. 2) DOCK2-deficient and control B cells expressing HEL-specific BCR were adoptively transferred into mice for in vivo functional assays. 3) The conditional KO mice lacking DOCK2 in B-cell lineage were developed and used to examine antigen-specific antibody production.Results:BCR-mediated Rac activation was almost completely lost in DOCK2-deficient B cells, resulting in defects in B cell spreading over the target cell-membrane and sustained growth of BCR microclusters at the interface. When wild-type B cells were stimulated in vitro with anti-IgM $F(ab')_2$antibody in the presence of cytokines, they differentiated efficiently into PCs. However, BCR-mediated PC differentiation was severely impaired in the case of DOCK2-deficient B cells. Similar results were obtained in vivo when DOCK2-deficient B cells expressing HEL-specific BCR were adoptively transferred into mice and challenged with the HEL antigen. In addition, by generating the conditional knockout mice, we found that DOCK2 expression in B-cell lineage was required to mount antigen-specific IgG antibody. Conclusion: The Rac activator DOCK2 regulates PC differentiation and antigen-specific IgG production.

P.A4.01.11

Transcription of the Ets1 gene is diminished in response to BCR signaling in an IKK2-dependent manner

***A. Kearly**, L. Garrett-Sinha;*
State University of New York at Buffalo, Buffalo, United States.

Introduction: The transcription factor Ets1 is required to retain B cells in a quiescent state. In response to BCR stimulation, Ets1 is downregulated in a manner dependent upon the kinase IKK2. However, whether this downregulation is due to changes in Ets1 gene transcription, Ets1 mRNA stability, and/or Ets1 protein stability is not known. It is also not clear whether NFκB is involved in downregulation of Ets1.
Methods: To determine whether Ets1 gene transcription is altered by activation, we measured newly-transcribed Ets1 precursor mRNA (pre-mRNA) by qPCR. This was complemented by assessing RNA polymerase II (Pol II) occupancy at the Ets1 promoter by ChIP-qPCR. To determine the role of NFκB in the downregulation of Ets1, we tested the effects of RelA (p65) loss using both B cell-specific RelA knockout mice and RelA siRNA knockdown.
Results: Levels of Ets1 pre-mRNA diminish in response to BCR stimulation in an IKK2-dependent manner. Pol II binding at the Ets1 promoter is also decreased with stimulation. Preliminary results further suggest that RelA is not required for this effect.
Conclusions: Control of Ets1 levels in B cells seems to be at the level of Ets1 gene transcription. Although the kinase IKK2 is required for this effect, RelA may not be a required downstream effector. Our studies are important because Ets1 is a key regulator of B cell quiescence and understanding how its expression is controlled may help develop therapies that can regulate B cell activation during immune responses.

P.A4.01.12

Temperature regulation of B cell activation

***M. LE BORGNE**[1,2], E. Procopio[1,2], B. Gachet[1,2], A. Loste[1,2], O. Thaunat[3,4], G. Caligiuri[1,2,5], A. Nicoletti[1,2];*
[1]INSERM U1148 "Laboratory for Vascular Translational Science", Paris, France, [2]Université Paris Diderot, Université Sorbonne Paris Cité, DHU FIRE, Paris, France, [3]INSERM U851, Lyon, France, [4]Hospices Civils de Lyon, Hôpital Edouard Herriot, Université de Lyon, Lyon, France, [5]Assistance Publique-Hôpitaux de Paris, Hôpital Bichat, Paris, France.

Elevated temperatures are often associated with the induction of immune responses: global elevation of body temperature in the case of fever, or local heat in the case of inflammation. It is known that elevated temperature impact the activation and functions of innate immune cells and T cells. However, the impact of temperature on B cell responses has been barely addressed.
In order to study if the activation and function of B cells are dependent on temperature, we stimulated B cells *in vitro* at different temperatures, and looked at the survival and activation of B cells by flow cytometry. We also analysed if activation of B cells with antigen-coated synthetic particulate antigens (SPAg) at different temperatures impacted their ability to later present antigens to antigen-specific T cells. We observed that elevated temperatures decreased the survival of B cells, and increased the internalization of CD19 (a member of the BCR co-receptor) and the upregulation of the costimulatory molecule CD86. B cells activated with SPAg at fever-like temperatures induced more proliferation of antigen-specific T cells in subsequent co-culture assays.
Altogether, our data show that activation of B cells depends on temperature. Further experiments are ongoing to decipher if temperature controls other aspects of B cell function *in vitro*, such as antibody production, and B cell activation *in vivo* in the context of vaccination.

P.A4.01.13

Size of CD40L signalling domain regulates efficacy of human naïve B cell differentiation and IgG class-switch recombination

C. Marsman[1], *P. Unger*[1], *N. Verstegen*[1,2], *T. Jorritsma*[1], *A. ten Brinke*[1], *M. van Ham*[1,3];
[1]*Sanquin Research, Dept Immunopathology, Amsterdam, Netherlands,* [2]*Synthetic Systems Biology and Nuclear Organization, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands,* [3]*Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands.*

Human naïve B cells are notoriously difficult to differentiate into antibody-secreting cells (ASCs) *in vitro* due to unknown regulatory mechanisms involved in this process. Insights in factors controlling differentiation of B cells into antibody-secreting plasmablasts (PB) and plasma cells (PC) however, are not only important to generate effective humoral immunity against invading pathogens, but also to prevent undesired antibody formation in autoimmunity and blood transfusion. After uptake of antigens *in vivo*, B cells require co-stimulatory signals, like CD40L, and cytokines, like IL-4 and IL-21, from cognate T follicular helper (T_{FH}) cells during the germinal center response to undergo PB/PC differentiation. Here we elucidated that the size of the CD40L signalling domain is key in inducing significant naïve B cell to ASC differentiation *in vitro.* Our data, using different sizes of fractionated CD40L-expressing cell membranes, show that the efficacy of *in vitro* IgG class-switch and differentiation of naïve B cells into ASCs are highly dependent on the size of the CD40L signalling domain and can be dramatically induced in the appropriate cytokine environment. We have unravelled how T_{FH} cytokines IL-21 and IL-4 and variation of CD40L co-stimulation regulate the kinetics of phosphorylation of various signal transducers and activators of transcription (STAT1, STAT3, STAT5 and STAT6) involved in PB/PC differentiation. Our data are the first steps to provide much needed insight in the process of human naïve B cell differentiation to ASCs. This is not only crucial in improving vaccination strategies but will also aid in the prevention and treatment of auto-immunity.

P.A4.01.14

DGKζ dependent PA production at the B cell immune synapse regulates antigen presentation and the B cell response.

S. Merino Cortes[1], *S. Gardeta Castillo*[1], *S. Roman Garcia*[1], *A. Martínez-Riaño*[2], *B. Alarcon*[2], *Y. R.Carrasco*[1];
[1]*Centro Nacional de Biotecnolgia, Madrid, Spain,* [2]*Centro de Biologia Molecular Severo Ochoa, Madrid, Spain.*

BCR recognition of antigen at the APC surface leads to immune synapse (IS) formation. Vinculin and Rac GEFs regulate actin cytoskeleton during IS assembly. Vinculin and Rac GEFs are recruited to the B cell IS. These events lead to Rac activation and F-actin polymerization. DGKζ(diacylglycerol kinase ζ) metabolizes the DAG generated following antigen recognition to produce PA (phosphatidic acid). In non-immune cells, DGKζ is involved in controlling PIP_2 levels, by PA-dependent allosteric modulation of PIP5KI, and Rac recruitment. A proper synapse assembly is necessary for antigen acquisition and B cell activation; the DGKζ role on these molecular and activation events is unknown. We used primary B cells from wild type and DGKζ-/- mice, non-treated or treated with the pan-DGK inhibitor R59. Our results suggest that DGKζ-dependent PA production regulates Vinculin and Rac GEFs recruitment as well as F-actin polymerization at the B cell IS. DGKζ also controls MTOC polarization to the synapse. The impaired IS structure of DGKζ-/- and R59-B cells contrasts with increased B cell activation, suggesting that DGKζ exerts a balance between DAG consumption and PA production. Analysis *in vitro* of the antigen presentation of DGKζ-/- and R59-B cells showed reduced T cell proliferation and IL-2 production, suggesting defects in B cell antigen acquisition. Moreover, adoptive transfer revealed a decrease in B cell differentiation to plasma cells and IgG1+ cells for DGKζ-deficient mice. Our studies highlight an important role for DGKζ in B cell IS structure that controls antigen up-take and presentation to T cells.

P.A4.01.15

microRNA-148a: regulator of plasma cell differentiation and maintenance

K. Pracht, *J. Meinzinger, P. Daum, J. Côrte-Real, M. Hauke, S. Schulz, E. Roth, J. Wittmann, H. Jäck;*
Division of Molecular Immunology, Department of Internal Medicine III, Nikolaus-Fiebiger Center, Uni, Erlangen, Germany.

microRNAs (miRNAs) are critical regulators of central and Ag-driven B cell development. In addition, several plasma cell (PC) associated-diseases and a variety of cancer types are caused by deregulated miRNA-expression. However, it is still unclear how single miRNAs regulate the formation and survival of healthy or malignant PCs. We showed that miR-148a, the most abundantly expressed miRNA in PCs, is upregulated in activated B cells and promotes *in vitro* plasmablast differentiation and viability by targeting Bach2, MiTF, PTEN and Bim. To determine wether miR-148a is involved in the maintenance of PCs we estalished a tamoxifen-inducile miR-148a deficient mouseline. Deletion of miR-148a, 4 weeks after boost imunisation, resulted in reduced numbers of mature PCs and dividing plasmabasts in the spleen. While the number of mature PCs was also reduced in the BM, their frequency was significantly elevated in the blood. Moreover, *in vitro* deletion of miR-148a, 3 days after stimulation, revealed diminished numbers of viable LPS blasts. ELISpot analysis of isolated miR-148a deficient PCs showed altered isotype composition in the spleen and the bone marrow. These findings support the hypothesis that miR-148a controls the formation of plasma blasts as well as the maintenance of long-lived PCs and could be a potential target for the treatment of PC-associated diseases. Supported by DFG grants GRK1660 and TRR130 to H.-M.J.

P.A4.01.16

A closer look at tissue-specific B cell regulation

J. H. Y. Siu[1], *Y. Zhao*[2], *T. J. Tull*[2], *K. T. Mahbubani*[1], *C. Pararasa*[2], *R. Ellis*[3], *N. Petrov*[3], *J. Spencer*[2];
[1]*University of Cambridge, Cambridge, United Kingdom,* [2]*King's College London, London, United Kingdom,* [3]*Biomedical Research Centre, Guy's and St Thomas' NHS Trust, London, United Kingdom.*

Lymphoid tissue is essential for normal B cell maturation and activation. Whilst B cell subsets in peripheral blood are well characterized, human tissue B cells and the differences that exist between blood and tissue are poorly understood.
We use mass cytometry (CyTOF) to perform a phenotypic analysis of B cell subsets in paired healthy human peripheral blood and gut-associated lymphoid tissue (GALT) (n=8). Spleen (n=6) and tonsil (n=5) were included for comparison. Using a panel of 34 antibodies to identify markers of B cell lineage, migration and activation, we assess their relative surface expression by B cell subsets across blood and tissue compartments.
We observe B cell surface phenotype alters dramatically as B cells enter tissues. In addition to changes in expression of chemokine receptors and homing markers, reduced expression of CD19 and IgD by naive B cells was observed in tissues compared to blood. This was confirmed in paired samples of blood and GALT by flow cytometry. Interestingly some differences that were observed between blood and GALT were not shared with other tissues. An example of this was reduced expression of CD40 by B cells in GALT compared to blood and other tissues suggesting that B cells may have reduced potential for CD40L dependent interactions with T cells in GALT. This study highlights that different tissues may regulate and modulate the potential for B cell responses in distinct ways.
Funded by UK Medical Research Council, and Biomedical Research Centre of Guy's and St Thomas' NHS Trust.

P.A4.01.17

Quantitative assays to measure human B lymphocyte health

J. C. Tempany[1,2], *V. L. Bryant*[1,2,3], *P. D. Hodgkin*[1,2];
[1]*The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia,* [2]*Department of Medical Biology, University of Melbourne, Melbourne, Australia,* [3]*Department of Clinical Immunology and Allergy, The Royal Melbourne Hospital, Melbourne, Australia.*

We have developed standardised, quantitative functional assays for human B-lymphocyte responses to T-dependent (CD40L+IL-21) and T-independent (anti-Ig±CpG) stimuli. These assays measure division, death, differentiation and isotype switching, to reveal the innate programming of B lymphocytes in response to these conditions.
Here, we observed, for the first time, regulation of the size of the division burst (division destiny) in human B cells, a phenomena shown to be essential for appropriate regulation of murine T- and B-lymphocyte responses. We also determined that human B lymphocytes regulate survival, independently of both division rate and division destiny. Thus, we propose that standardised quantitative assays, and accompanying parametric models, can provide a sensitive measure of the 'health' of B cells, and may reveal underlying B cell dysfunction in patients with monogenic or complex immune disorders.
As a first test of this hypothesis we focused on patients with Common Variable Immunodeficiency (CVID), the most prevalent primary immunodeficiency. CVID is a clinically heterogenous disorder, united by antibody deficiency, where most cases are sporadic and, presumably, polygenic. We hypothesised that for many CVID cases, the sum of multiple small changes in cellular functions are responsible for an antibody deficit when combined in an immunodeficient individual. In a preliminary screen of 12 patients, we have identified defects in various parameters of B cell responses including survival, division, differentiation, isotype switching and immunoglobulin production. We are now using this approach to test B cell responses in patients from multiplex families to investigate combinatorial changes in cellular functions in affected individuals.

P.A4.01.18

A novel multidisciplinary approach to elucidate disease causing processes in the germinal center

P. A. van Schouwenburg[1], *S. Unger*[2,3], *I. Pico-Knijnenburg*[1], *F. N. Kaiser*[1], *J. Pfeiffer*[4], *O. Hausmann*[5], *D. van Zessen*[6], *A. P. Stubbs*[6], *M. van der Burg*[1], *K. Warnatz*[2];
[1]*Department of Immunology, Erasmus MC University Medical Center, Rotterdam, Netherlands,* [2]*Center for Chronic Immunodeficiency (CCI), Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany,* [3]*University of Freiburg, Faculty of Biology, Freiburg, Germany,* [4]*Department of Otorhinolaryngology- Head and Neck Surgery, University of Freiburg, Freiburg, Germany,* [5]*Löwenpraxis and Klinik St. Anna, Zürichstrasse12, 6004, Luzern, Switzerland,* [6]*Clinical Bioinformatics Unit, Department of Pathology, Erasmus MC University Medical Center, Rotterdam, Netherlands.*

Here, we present a novel multidisciplinary approach to analyse patient germinal center (GC) material to further elucidate disease causing processes in individual patients. By combining histology, flow cytometry and B-cell receptor repertoire analysis of sorted GC B-cell populations we are able to model the disturbances in different patients.
Currently we have analysed three patients suffering from Common Variable Immune Deficiency disorder (CVID), a highly diverse disease characterized by recurrent infections, low IgG levels with low IgA and/or IgM and poor vaccination responses. We have previously found that CVID patients often have a defect in the GC.
Our data show that all three patients have different defects in the GC. In one patient both quantitative and qualitative B-cell development is normal in the GC, but only little memory B-cells are present in the periphery. In both other patients, GCs are non-polarised and abnormally shaped. Analysis in the second patient suggests impaired induction of SHM, poor antigen selection and impaired class-switching. IgM plasmablasts and IgM and IgG memory B-cells are formed but qualitative defective, while IgG plasmablasts are qualitatively normal but reduced in numbers. Results in the final patient indicate increased cycling of cells in the GC producing plasmablasts with increased SHM in the GC, and in very limited numbers in the periphery. Antigen selection and CSR are also impaired.
In addition to giving new insights in the GC reaction in CVID, this approach can also be applied to other immunological diseases with presumed GC defects.

P.A4.01.19

Do neonates benefit from influenza vaccination in the presence of high levels of maternal antibodies?

M. Vono[1], *C. Eberhardt*[1], *M. Schmolke*[2], *D. Christensen*[3], *P. Andersen*[3], *P. Lambert*[1], *C. Siegrist*[1];
[1]*WHO Collaborative Center for Vaccine Immunology, Department of Pathology and Immunology,University of Geneva, Geneva, Switzerland,* [2]*Department of Microbiology and Molecular Medicine, University of Geneva, Geneva, Switzerland,* [3]*Vaccine Adjuvant Research, Department of Infectious Disease Immunology, Statens Serum Institut, Copenhagen, Denmark.*

Influenza causes severe illness and death in high risk populations, including neonates and young infants, and remains a major public health issue. No influenza vaccine is currently licensed for use below 6 months of age. Maternal vaccination and transfer of vaccine-induced antibodies is an effective alternative - but with a transient efficacy. In our murine model, naïve pups from immunized mothers born with high levels of influenza-specific maternal antibodies (5 log10) loose protection against challenge when their antibody titers reach 3 log10 or less. Vaccination is the best way to prevent infection and we recently identified the adjuvant CAF01 as the first adjuvant able to induce germinal centers and protective primary responses to influenza hemagglutinin (HA) in neonatal mice. Here, we asked whether HA/CAF01 immunization at the age of 1-week remains effective in presence of maternal antibodies. Unexpectedly, total $CD4^+$ T cell, T follicular helper cell and even Germinal Center (GC) B cell responses to HA/CAF01 in neonates were preserved in the presence of maternal antibodies. Organized GC structures were observed even in the presence of high levels of maternal antibodies. However, these GCs did not generate effector functions: plasma cells, antibody and memory B cell responses to HA/CAF01 were fully inhibited. Although present, HA-specific T cell responses failed to protect mice against a lethal influenza virus challenge when maternal antibodies were no longer sufficient. Thus, the protective efficacy of neonatal HA/CAF01 vaccination is lost in the presence of high levels of maternal antibodies and alternative vaccination strategies are needed.

P.A4.01.20

$CD4^+$ T cell-released exosomes potentiate the HBsAg Vaccine by enhancing B cells respons

J. Lu[1,2], *J. Wu*[3], *H. Xu*[3], ***S. WANG***[1,2];
[1]*Department of Laboratory Medicine, The Affiliated People's Hospital, Jiangsu University, ZHENJIANG, China,* [2]*Department of Immunology, School of Medicine, Jiangsu University, Zhenjiang, China,* [3]*Department of Immunology, School of Medicine, Jiangsu University, ZHENJIANG, China.*

Introduction: $CD4^+$ T cells excrete bioactive exosomes, but the potential biological effect of CD4+T-cell exosomes is still unclear. The principal objective of this research was to investigate the role of the CD4+T-cell exosomes on antibody production and B cells activation when challenged with HbsAg.
Materials and Methods: In this study, $CD4^+$T-cell exosomes were purified from in vitro activated $CD4^+$ T cells. The role of $CD4^+$T-cell exosomes were administrated in HBsAg vaccinated mice, and B cells responses were evaluated in vitro.
Results: After immunized with commercial HBsAg, $CD4^+$T-cell exosomes treated mice showed a stronger humoral immune response, as reflected by higher specific antibody levels in serum and higher proportion of plasma cells in bone marrow. In vitro experiment, we found that exosomes derived from activated $CD4^+$T cells both promoted B cells activation and proliferation. In addition, antigen-specific exosomes shows stronger biological function than normal exosomes. Using mouse T cell line EL-4, we showed that CD40L plays a significant role in $CD4^+$T-cell exosomes mediated B cells responses.
Conclusions: Taken together, CD4+T-cell exosomes can promote B cell activation in vitro and be served as a novel adjuvant to promote mouse antigen-specific humoral immune responses.

P.A4.01.21

Galectin-9 Compromised T-dependent Antibody Production

Y. Wu[1], *F. Liu*[2], *K. Lin*[1];
[1]*Genomics Research Center, Academia Sinica, Taipei, Taiwan,* [2]*Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.*

Galectins are Ca^{2+}-independent animal lectins with binding specificity for β-galactosides. Galectin-9 is a tandem repeat-type galectin with two carbohydrate binding sites and a linker peptide. Previous studies demonstrated various regulatory roles of galectin-9 in the immune responses. Especially, it has been shown that galectin-9 might bind to Tim-3 and trigger the apoptosis of T helper type 1 cells. On the other hand, the role of galectin-9 in B cells remains elusive. Here, we observe normal B cell development in galactin-9 deficient mice. We found that the expression of galectin-9 was decreased in germinal center B cells (GC B cells) as compared with naïve B cells in either mice immunized with a T-dependent antigen, NP-KLH, or splenic B cells given T follicular helper (Tfh) derived stimuli *ex vivo*. The basal serum levels of IgA and IgG3 in galectin-9 deficient mice were also reduced. We then examined the immune responses following NP-KLH immunization in mice and found that NP-specific IgG production was reduced in galactin-9 deficient mice as compared with littermate control mice. Further, the population of GC B cells in immunized mice was significantly decreased in galactin-9 deficient mice. Given that the activation of B cells from galectin-9 deficient mice was comparable with that of wild type B cells, we suspect that galectin-9 might serve as a pivotal regulator in adaptive immunity via affecting T cell activation. We are examining the effect of lack of galectin-9 on T cell activation.

P.A4.01.22

T cell regulation of gut IgA responses

I. Gribonika, *K. Schön, N. Lycke;*
Depatment of Microbiology and Immunology, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden.

Understanding how gut IgA is induced is critical for the development of oral vaccines. We made the unexpected observation that adjuvant is not required for ovalbumin (OVA) -specific TCR-transgenic (Tg) mice to respond strongly with IgA plasma cells in the *lamina propria* (LP) after oral immunization. Adoptive transfer of TCR Tg CD4 T-cells into nu/nu mice in the absence of adjuvant demonstrated that the IgA response in this context is T cell dependent. Importantly, we revealed the transferred CD4 T-cells exhibited a rearranged α-chain and expressed a dual TCR phenotype. We identified that microbiota recognition via second TCR was an important inducer of the response.
Interestingly, TGFβ1 signalling was required not only for authentic IgA class-switch recombination, but also for control of suppressive T follicular regulatory cells. We speculate that TGFβ1 was thymic T regulatory cell (tTreg) derived as regulatory cell depletion from total CD4 T cell pool prior to adoptive transfer in athymic mice resulted in the absence of OVA-specific IgA, but not IgG antibodies. This suggests that follicular helper function can be distinguished from class-switching in Peyer's patches by T follicular helper cells and tTregs respectively. Together these findings illustrate the complex nature of T-dependent IgA induction at mucosal sites.

P.A4.01.23
Role of Grail in B cell activation and tolerance

*S. Bieerkehazhi[1], T. C. Waseem[2], A. Alekseev[1], E. V. Galkina[2], **R. Nurieva**[1];*
[1]MD Anderson Cancer Center, Houston, United States, [2]Eastern Virginia Medical School, Norfolk, United States.

To date, B cells are believed to play a central role in the pathogenesis of various autoimmune diseases. Loss of B-cell tolerance with emergence of autoreactive B cells and pathogenic autoantibodies are the hallmark features of autoimmune disorders. However, the intrinsic mechanisms that underlie initial disruptions in B cell tolerance have not been completely defined. Grail, gene related to anergy in lymphocytes (encoded by Rnf128) is an E3 ubiquitin ligase associated with $CD4^+$ and $CD8^+$ T cell tolerance. Our data for first time show Grail expression in both mouse and human B cells, with higher expression particularly in anergic B cells.
Grail deficiency in B cells lead to impaired B cell peripheral tolerance induction and greater susceptibility to autoimmune diseases. Grail deficient B cells were hyperresponsiveness in terms of proliferation and antibody production upon antigen stimulation *in vitro* and *in vivo*. Concomitantly, Grail-deficient B cells were less efficient in downregulation of IgM after B cell receptor (BCR) crosslinking and exhibited elevated activation/expression of BCR-signaling components and Ca^{2+} mobilization. Thus, our results indicate that Grail is a crucial intrinsic factor controlling B cell activation and tolerance by targeting of BCR signaling components.

P.A4.02 Germinal centers and B cell differentiation - Part 2

P.A4.02.01
Different epigenetic marks on *PRDM1* gene promoters determine the expression of the isoforms in human myeloma cells

*R. Romero García, L. Gomez-Jaramillo, F. Mora-Lopez, **A. Campos-Caro**;*
Hospital Universitario Puerta del Mar, Cádiz, Spain.

The human positive regulatory domain I (PRDM1) transcription factor, is considered the main regulator of terminal differentiation process from B-cells towards PCs. It is considered, in general, as a transcriptional repressor that silences several genes related to B-cell phenotype. In malignant PCs *PRDM1* gene has been described to originate two isoforms, PRDM1α and PRDM1β, by alternative transcriptional promoters. PRDM1β, which lacks the amino-terminal 101 amino acids compared to the normal PRDM1α, shows a loss of repressive function on multiple targeted genes, acting as a competitive dominant negative. Here we assessed if the methylation status as well as the histone modifications of the *PRDM1* gene promoters in myeloma PCs (MM-PCs) are related to the expression of the PRDM1α and PRDM1β isoforms. Normal-PCs and malignant MM-PCs were isolated from bone marrow samples. Human cell lines were also used. Methylation status of the PRDM1α and PRDM1β promoters was evaluated by bisulfite sequencing. Cell cultures were also treated with 5-dAza and ChIP assays were performed in order to investigate changes in transcriptional activity of *PRDM1* gene. Specific CpGs positions into the of the PRDM1α and PRDM1β promoters probably determine the turn-on/off transcriptional activity isoforms of the PRDM1 gene. On this way, 5´Aza treatment augmented the expression level of PRDM1 transcripts. On the other hand, ChIPs assays showed different histone marks when we compare the PRDM1 promoters. The loss/gain of epigenetic marks in the PRDM1 promoters contributes to modify the expression level ratio between PRDM1α and PRDM1β and this, consequently, might contribute to myeloma progression.

P.A4.02.02
The transcriptional profiling of human in vivo-generated plasma cells identifies selective imbalances in monoclonal gammopathies

*L. M. Valor, B. Rodríguez-Bayona, A. B. Ramos-Amaya, J. A. Brieva, **A. Campos-Caro**;*
Hospital Universitario Puerta del Mar, Cádiz, Spain.

Plasma cells (PC) represent the heterogeneous final stage of the B cells (BC) differentiation process. To characterize the transition of BC into PC, transcriptomes from human naïve BC were compared to those of three functionally-different subsets of human in vivo-generated PC: i) tonsil PC, mainly consisting of early PC; ii) PC released to the blood after a potent booster-immunization (mostly cycling plasmablasts); and, iii) bone marrow CD138+ PC that represent highly mature PC and include the long-lived PC compartment. This transcriptional transition involves subsets of genes related to key processes for PC maturation: the already known protein processing, apoptosis and homeostasis, and of new discovery including histones, macromolecule assembly, zinc-finger transcription factors and neuromodulation. This human PC signature is partially reproduced in vitro and is conserved in mouse. Moreover, the present study identifies genes that define PC subtypes (e.g., proliferation-associated genes for circulating PC and transcriptional-related genes for tonsil and bone marrow PC) and proposes some putative transcriptional regulators of the human PC signatures (e.g., OCT/POU, XBP1/CREB, E2F, among others). Finally, we also identified a restricted imbalance of the present PC transcriptional program in monoclonal gammopathies that correlated with PC malignancy.

P.A4.02.03
Stromal cell contact-induced PI3K signaling and BCMA-induced NF-κB signaling synergize to maintain plasma cells alive in the bone marrow

***R. Cornelis**, S. Hahne, H. D. Chang, A. Radbruch;*
German Rheumatism Research Centre (DRFZ Berlin), a Leibniz Institute, Berlin, Germany.

Long-lived memory plasma cells (mPC) can survive for years in niches organized by mesenchymal stromal cells in the bone marrow. We have developed an *in vitro* niche using ST2 stromal cells and the cytokine APRIL, under hypoxic conditions, to maintain mPC alive *ex vivo*. Here we show for the first time that direct cell contact between stromal cells and mPC is required for the mPC to survive. Apparently, cell contact induces PI3K signaling, while APRIL is known to induce NF-κB signaling. Both, cell-contact dependent PI3K and APRIL-induced NF-κB signaling are required and sufficient for mPC survival. Inhibition of either pathway kills mPC in the *in vitro* niche, as well as *in vivo*, in the bone marrow. PI3K and NF-κB signaling in synergy upregulate IRF4, a transcription factor critical for mPC survival, and the ratio of MCL1 to NOXA, and of BCL2 to BIM, critical for the prevention of caspase-dependent apoptosis. PI3K signaling downregulates expression of all four BCL2-family members. NF-κB signaling rescues the expression of the anti-apoptotic proteins BCL2 and MCL1, shifting the ratio of pro- versus anti-apoptotic proteins in favor of the latter. PI3K signaling also downregulates FoxO1 and FoxO3, probably mediating survival by downregulating expression of the pro-apoptotic proteins NOXA and BIM. The stromal cell contact dependent survival of memory plasma cells in the bone marrow may serve as a paradigm for maintenance of tissue-resident memory cells in general, since inhibition of PI3K also ablates memory T and memory B cells of the bone marrow.

P.A4.02.04
IgG4-related disease of the skull base in two patients with normal serum IgG4

***S. E. Detiger**[1], A. F. Karim[2,3], J. van Laar[2];*
[1]The Rotterdam Eye Hospital, Rotterdam, Netherlands, [2]Departments of Internal Medicine and Immunology, section Clinical Immunology, Erasmus Medical Center, Rotterdam, Netherlands, [3]Department of Internal Medicine, Groene Hart Hospital, Gouda, Netherlands.

Introduction: IgG4-related disease (IgG4-RD) is an immune-mediated systemic fibro-inflammatory disease, which may mimic a variety of disorders. The pathogenesis is mostly unclear, but B cells, IgG4 positive plasma cells, IgG4 antibodies, as well as the oligoclonal expansion of T cells seem to play an important role in the immunopathophysiology of IgG4-RD. IgG4-RD may manifest in almost every part of the human body. Here, we describe two patients with skull base manifestation of IgG4-RD that mimicked nasopharyngeal cancer. Case presentations: Patient 1, a 73-year-old male, with a history of smoking, diabetes mellitus type 1 and vascular disease, presented with a mass in the left nasopharynx extending to the cavernous sinus. Patient 2 was a 74-year-old male with a history of chronic obstructive pulmonary disease (COPD) and colon cancer who presented with a mass extending from the left nasopharynx into the inner ear with involvement of the left jaw joint. Both patients complained of pain and hearing loss. In both cases, serum IgG4 was normal and imaging did not show systemic manifestation of the disease. However, histology confirmed the diagnosis of IgG4-RD. Patients are currently treated successfully. Discussion: The described cases emphasize the broad clinical spectrum of IgG4-RD. The diagnostic workup may be challenging and serum IgG4 may be normal, as demonstrated in these cases. Careful histopathological examination of the tissues remains essential. Timely diagnosis of IgG4-RD is important to prevent secondary organ damage. Conclusion: IgG4-RD is a systemic disease and may present with normal serum IgG4.

P.A4.02.05

T cells modulate B cell receptor signaling in mature antigen-naive B cells via CD40L

***R. W. Hendriks**, J. Rip, M. Appelman, M. De Bruijn, H. Hau, O. Corneth;*
Erasmus MC Rotterdam, Rotterdam, Netherlands.

Survival of mature peripheral B lymphocytes requires B cell receptor (BCR) signaling in the absence of exogenous antigen-binding. However, the relationship between tonic BCR signals and antigen-triggered signals is currently unknown. Also, the mechanisms by which T-cell derived signals contribute to B cell survival remain unexplored. B-cell-specific overexpression of the BCR signaling molecule Bruton's tyrosine kinase (BTK) induces spontaneous germinal center formation, autoantibodies and systemic autoimmunity. In aging BTK-transgenic mice naive B cells manifest increased IFNγ, IL-6, IL-10 and surface CD86 expression. Except for increased IL-10, this phenotype of naive B cells was dependent on B-T cell interaction, because it was lost in CD40L-deficient BTK-transgenic mice. To further investigate the role of CD40-CD40L interaction on B cell responsiveness, we compared BCR signaling in naive splenic B cell subsets from CD40L-deficient and wild-type mice. We used intracellular flowcytometry to study phosphorylation of various BCR signaling proteins including PLCγ, as well as rS6, which is downstream of the AKT pathway. Consistent with the *in vivo* pre-activated state of marginal zone (MZ) B cells, we found that in the absence of antigen-stimulation the levels of phospho-PLCγ and phospho-rS6 were higher in MZ B cells than in immature or follicular B cells. Hereby, no differences between CD40L-deficient and wild-type mice were observed. Upon BCR stimulation phospho-PLCgamma and phospho-rS6 increased in wild-type follicular B cells and particularly in MZ B cells, but these responses were lower in CD40L-deficient mice. Therefore, we conclude that CD40 signaling modulates BCR responsiveness in mature antigen-naive splenic B cells.

P.A4.02.06

Functional consequence of atypical B cells for*in vivo*development of antiviral B cell responses in patients with hemorrhagic fever with renal syndrome

***P. F. Kerkman**[1], A. Håglin Dernstedt[1], R. Jangra[2], A. Tuiskunen-Bäck[1], J. Wigren Byström[1], K. Maleki[3], J. Tauriainen[3], J. Klingström[3], K. Chandran[2], C. Ahlm[1], M. N. Forsell[1];*
[1]Umeå University, Umeå, Sweden, [2]Albert Einstein College of Medicine, New York, United States, [3]Karolinska Institutet, Stockholm, Sweden.

Circulating B cells in healthy individuals comprise a small fraction of CD27-IgD- atypical B cells (ABCs). These cells may accumulate in patients with Systemic Lupus Erythematosus (SLE) nephritis. *In vitro* studies imply that ABCs are dysfunctional or exhausted but their *in vivo* biological function remains poorly understood. Hantavirus infections that cause hemorrhagic fever with renal syndrome (HFRS) lead to transient kidney dysfunction in patients, as shown by increased serum creatinine levels. We hypothesized that development of ABCs is associated with reduced kidney function, and that studies of HFRS could be used to assess if accumulation of ABCs is detrimental to the development of antiviral humoral immunity.
Using longitudinal HFRS-patient samples stratified based on the median creatinine level, we demonstrate that ABCs accumulate in circulation of high creatinine patients but not in patients with lower serum creatinine levels. Phenotypical analysis showed that HFRS-induced ABCs have lower expression of activation markers and show reduced capacity for antigen presentation to T cells. Moreover, we found that ABCs have lower expression of a complement regulatory protein, indicative of complement deposition. To assess the impact of ABCs on functional immune responses, we are characterizing longitudinal capacity of patients to mount antibodies that bind specifically and/or neutralize the homologous Hantavirus strains.
Collectively, this study demonstrates an association between reduced kidney function and accumulation of ABCs in circulation. Moreover, our data shed light on the potential impact that accumulation of circulating ABCs may have on productive antiviral responses in HFRS-patients.

P.A4.02.08

A computational model of kinetic maturation in the germinal center

***D. Lashgari**[1], M. Meyer-Hermann[2], R. W. Sanders[3], M. J. van Gils[3], A. H. van Kampen[1];*
[1]Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, Amsterdam, Netherlands, [2]Department of Systems Immunology and Braunschweig Integrated Centre of Systems Biology, Helmholtz Centre for Infection Research, Braunschweig, Germany, [3]Department of Medical Microbiology, Academic Medical Center, Amsterdam, Netherlands.

Germinal centres (GC) are sites of affinity maturation, an evolutionary process in which B lymphocytes proliferate, undergo somatic hypermutations (SHM) and positive selection to produce high-affinity antibodies (Ab) eventually. Ab affinity (K_A) is defined as the ratio of kinetic constants k_{on} and k_{off} which determine the kinetics of the bond between B-cell receptors (BCR) and antigens (Ag) and between peptide-MHCs (pMHC) and T follicular helper (T_{FH}) cells. We aim to investigate the dependency of spatiotemporal dynamics, affinity maturation, and output of the GC on individual contributions of kinetic
constants. The model will be based on experimentally measured kinetic constants and affinities. We will extend a pre-existing agent-based model (ABM) of the GC (Meyer-Hermann et al., 2012) that comprises of the primary cellular mechanisms of the GC reaction (e.g., cell movement, B-cell proliferation, differentiation and apoptosis, SHM, Ag binding, positive B-cell selection). Binding kinetics will replace affinity representation in this model. We are currently implementing kinetics maturation in the ABM. We use three different scenarios to modify BCR-Ag binding kinetics since it is still not clear how k_{on} and k_{off} change during a GC reaction. It is assumed that k_{off} is altered through SHM in all scenarios while k_{on} is considered constant, affected by cell motility or affected only by SHM in separate scenarios. By comparing the output of these models to in-house generated experimental data and data from published studies, we aim to define the kinetics of GC reaction in more details.

References

Meyer-Hermann et al. (2012), *Cell Rep,* 2(1):162-174.

P.A4.02.09

Multiscale modeling of plasma cell differentiation in germinal centers

***E. Merino Tejero**[1], X. Gao[2], P. A. Robert[3], M. R. Martinez[4], F. Crauste[5], O. Gandrillon[5], M. M. Hermann[6], H. C. Hoefsloot[7], J. Guikema[8], A. V. Kampen[9];*
[1]Amsterdam Medical Center, Amsterdam, Netherlands, [2]Chinese Academy of Sciences, Beijing, China, [3]Department of Systems Immunology and Braunschweig Integrated Centre of Systems Biology, Helmholtz Centre for Infection Research, Braunschweig, Germany, [4]IBM Zurich Research Laboratory, Zurich, Swaziland, [5]Inria team Dracula, Lyon, France, [6]Department of Systems Immunology and Braunschweig Integrated Centre of Systems Biology, Helmholtz Centre for Infection Research; Institute for Biochemistry, Biotechnology and Bioinformatics, Technische Universität Braunschweig, Braunschweig, Germany, [7]Swammerdam Institute for Life Sciences, University van Amsterdam, Netherlands, [8]Department of Pathology, Academic Medical Center, Amsterdam, Netherlands, [9]Bioinformatics Laboratory, Amsterdam Medical Center, Amsterdam, Netherlands.

B cells undergo affinity maturation within the GCs forming high affinity Ab producing plasma and memory cells. The GC comprises complex interacting temporal and spatial dynamic processes at the cellular level (e.g., moving and interacting cells) and molecular level (e.g., interacting proteins in gene regulatory networks; GRNs). To understand potential mechanisms involved in health and disease (e.g., B-cell lymphoma) novel methods are urgently needed, such as multiscale modeling (MSM), that integrate these levels (Gao, X. *et al.*, 2016). We aim to develop a MSM that integrates the GC cellular dynamics with a GRN representing plasma cell differentiation. The MSM contributes to a better understanding of plasma cells differentiation in GCs and the mechanisms involved in this process. Furthermore, we will be able to predict changes in cell populations originating from perturbations in the dynamics of GRNs. An existing agent-based model (ABM) of the GC is used to represent the cellular level (Meyer-Hermann *et al.*, 2012). It describes B-cell behaviours in the GC including proliferation, SHM, selection and differentiation. The GRN is based on an existing model comprising ordinary differential equations (ODE; Martinez *et al.*, 2012). In the MSM we effectively embed the GRN (ODEs) in each B-cell represented by the ABM. One of the main challenges is to link the parameters of the cell-based model (e.g., proliferation, differentiation, apoptosis rates) with parameters of the GRN (e.g., kinetic constants, BCR signal, CD40 signal). Currently, we are calibrating the model to experimental data to lay the basis for making realistic predictions.

P.A4.02.10

Siglec-G deficiency leads to autoimmunity and earlier onset of chronic lymphatic leukemia

*L. Özgör, S. Mrotzek, M. Korn, H. Fahnenstiel, **L. Nitschke**;*
University of Erlangen, Erlangen, Germany.

Siglec-G is an inhibitory receptor on B cells. Siglec-G deficient mice show increased Ca2+ signalling particularly in B1 cells and a large B1 cell expansion. Furthermore, ageing Siglec-G deficient mice develop a lupus-like autoimmune disease. Higher activation of B cells as well as dendritic cells may contribute to this autoimmune disease. We have previously shown, that CD22 (Siglec-2) x Siglec-G double deficient mice develop a stronger autoimmune disease than Siglec-G deficient or CD22-deficient mice. One hypothesis to explain these data was that these two Siglecs bind to their ligands, sialic acids in specific linkages and this controls B cell tolerance, as sialic acids are abundantly expressed self ligands. However, we could show in a new mouse model in which both Siglecs are defective in ligand binding, that this is not the case, as these mice do not develop autoimmune disease.Chronic lymphatic leukemia (CLL) is derived from CD5+ B cells. CD5+ B1a cells are largely expanded in Siglec-G deficient mice. We could show that these mice develop much earlier and stronger CLL in the Tcl1-transgenic mouse model.

P.A4.02.11

The Art2.2/P2X7-system differently affects functionality of murine CD4 T and iNKT subsets

***G. Papadogianni**, H. Georgiev, I. Ravens, G. Bernhardt;*
Hannover Medical School, Hannover, Germany.

Introduction: P2X7 represents an ATP-gated ion channel promoting inflammasome formation and T cell activation. P2X7 can be locked in an open stage following ADP-ribosylation by the ecto-enzyme ART2.2 thereby triggering induction of apoptosis. We investigated the impact of the ART2.2/P2X7-system on functionality of iNKT and CD4 T cell subsets isolated from various organs.
Materials and Methods: Cell suspensions of thymus, spleen, Peyer's Patches (PP) and peripheral lymph node (pLN) with or without prior immunization were prepared and incubated in presence or absence of the drug KN-62 blocking P2X7. Cells were then analyzed by flow cytometry to determine apoptosis and cytokine production.
Results: The expression patterns of P2X7 and ART2.2 correlated well with the observed degree of apoptosis. Whereas naïve CD4 T cells resisted induction of apoptosis, follicular T cells were rather sensitive yet regulators were less affected than helpers. CD4 cell subsets of PP origin were more sensitive than their corresponding counterparts from pLN. Moreover, thymic but not splenic iNKT cells resisted P2X7-triggered apoptosis. Presence of KN-62 during cell preparations and subsequent steps protected cells from apoptosis and also preserved their fitness to produce cytokines.
Conclusions: The highly diverging and organ dependent sensitivities of immune cells to apoptosis caused by P2X7 can warp readouts of experiments. This can be avoided by KN-62 application.
Funding was provided by DFG grants BE1886-5/1 and BE1886-7/1.

P.A4.02.12

Characterisation of the S1P receptor expression and of the functional role of S1P signalling in peritoneal B cells

*A. Kleinwort[1], F. Lührs[1], C. Heidecke[1], M. Lipp[2], **T. Schulze**[1];*
[1]Department of General Surgery, Visceral, Thoracic and Vascular Surgery, Greifswald, Germany, [2]Max-Delbrück-Centre for Molecular Medicine, Berlin, Germany.

Sphingosine-1-phosphate (S1P) regulates migration of follicular B cells (B2 cells) and directs the positioning of Marginal Zone B cells (MZ B cells) within the spleen. The function of S1P signalling in the third B cell lineage, B1 B cells, mainly present in the pleural and peritoneal cavity has not yet been analysed. Methods: S1P receptor expression was determined in peritoneal B cells by RT-PCR. The chemotactic response to S1P was studied in vitro. In order to assess the involvement of the individual S1P receptors expressed on peritoneal B cells, a specific S1P1 antagonist and/or cells from $s1p_4^{-/-}$ mice were used. Results: Peritoneal B cells expressed significant amounts of the S1P receptors 1 and 4 ($S1P_1$ and $S1P_4$, respectively). S1P1 showed differential expression. While B2 cells showed no chemotactic response to S1P, B1b B cells showed the most important migratory response. $s1p_4^{-/-}$ mice showed significant alterations of the composition of peritoneal B cell populations as well as a significant reduction of mucosal IgA in the gut. Interestingly, $s1p_4^{-/-}$ mice had significantly higher plasmatic IgA levels than wildtype animals, while IgM levels were similar. Discussion: S1P signalling influences peritoneal B1 B cell migration. $S1P_4$ deficiency alters the composition of peritoneal B cell populations and reduces secretory IgA levels. These findings suggest that S1P signalling may be a target to modulate B cell function in inflammatory intestinal pathologies.

P.A4.02.14

Signaling requirements for metabolic reprogramming of human B cells following TLR stimulation

***R. Steiner**, G. Bantug, S. Wiedemann, C. Kunz, C. Hess;*
Univ. Hospital Basel, Basel, Switzerland.

B cell intrinsic TLR signaling is important for successful control of invading microbes via generation of pathogen specific antibodies and in promoting immune tolerance by generation of regulatory B cells. The aberrant activation of TLRs on B cells can lead to various disorders from primary immune deficiencies to autoimmunity. TLR stimulation alone is sufficient in driving B cell activation and metabolic reprogramming. However, it remains unresolved, which signaling pathway drives metabolic rewiring in these cells. To examine TLR-dependent metabolic reprogramming in B cells, we sorted naïve (IgD^+CD27^{neg}) and memory ($CD27^+$) cells by and stimulated cells with the TLR9 agonist, CpG. Using a snapshot metabolomics and transcriptomics approach, we established that naïve and memory human B cells present distinct metabolic signatures following stimulation with CpG. To further examine metabolic changes in both B cell subsets, we monitored glycolysis and mitochondrial respiration by metabolic flux analysis. CpG was found to rapidly induced glycolysis in both subsets, whereas, mitochondrial respiration remained stable early after stimulation. Stimulation with CpG for 48 hours induced a marked enhancement of aerobic glycolysis and mitochondrial respiration in both B cell subsets. Early and late enhancements in glycolytic and mitochondrial respiration in naïve cells but not in memory B cells were primarily dependent on NF-kB signaling. Lastly, blockade of metabolic reprogramming using IKK1/2 inhibitors differentially impacted CpG induced cell activation, cytokine production, and early antibody production in both subsets. These results indicate that NF-kB dependent metabolic reprogramming and effector maturation are intricately linked in TLR stimulated B cells.

P.A4.02.15

Elucidation of the *in vitro* requirements for the generation of plasma cells from human naïve B cells

***P. Unger**[1], T. Jorritsma[1], M. Aalbers[1], A. ten Brinke[1], T. Rispens[1], M. van Ham[1,2];*
[1]Sanquin Research, Department of Immunopathology, Amsterdam, The Netherlands, and Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands, [2]University of Amsterdam, Swammerdam Institute for Life Sciences, Amsterdam, Netherlands.

CD40 co-stimulation of B cells by CD40L-expressing follicular T helper (T_{FH}) cells and T_{FH} cytokines is crucial for the generation of high-affinity antibodies. Whereas human memory B cells easily differentiate into antibody-secreting plasmablasts ($CD27^+CD38^{hi}$) upon CD40 and IL-21 stimulation *in vitro,* naïve B cells do not. *In vivo* plasmablast differentiation occurs after repeated contact with T_{FH} cells in cyclic germinal centre reactions, but the mechanisms underlying these observations remain largely unknown. Progressive T_{FH} differentiation shows a switch from IL-21$^+$ T_{FH} cells into IL-4$^+$ T_{FH} cells in mice. Furthermore, CD40L expression on T_{FH} cells is subject to dynamic regulation. Here, we elucidated the minimal co-stimulation and cytokine requirements for human naïve B cell differentiation into plasmablasts (PBs) and plasma cells (PCs). We investigated *in vitro* whether strength of CD40 co-stimulation in the presence of IL-4 and/or IL-21 regulates naïve B cell differentiation into PBs and PCs and whether repeated co-stimulation is an intrinsic requirement for differentiation. Variation of CD40L stimulation by CD40L-expressing 3T3 fibroblasts showed that strength of CD40 co-stimulation in presence of IL-21 was decisive for induction of the PB/PC transcriptional program (i.e. strong expression of *Prdm1* mRNA), while repeated co-stimulation was a key requirement to allow full effector PB and PC differentiation. The elucidation of the requirements to induce human naïve B cell differentiation into PCs *in vitro* now allow the investigation of the various steps in the human PC differentiation process and the discovery of targets to modulate desired and undesired antibody responses in vaccination, auto-immunity and allergy.

P.A4.02.16

Secretome screening reveals novel B cell differentiation factors

***S. D. van Asten**[1,2], P. Unger[1,2], S. Bliss[1,2], T. Jorritsma[1,2], C. Marsman[1,2], N. Makazaji[1,2], S. M. van Ham[1,2], R. M. Spaapen[1,2];*
[1]Dept. of Immunopathology, Sanquin Research, Amsterdam, Netherlands, [2]Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands.

The most important function of B cells is the production of pathogen-specific antibodies. B cells become activated upon antigen encounter and may differentiate into memory B cells or antibody secreting plasma cells. Known pro-survival and (co-)activation signals include CD40L co-stimulation and the cytokines IL-4 and IL-21. Since these signals are essential but not sufficient for full human B cell differentiation *in vitro*, we set up a secretome screen to identify novel factors for B cell differentiation. We tested the differentiating capacity of 756 secreted proteins on human naive or memory B cells supported by CD40L expressing cells and suboptimal amounts of IL-21. After 9 days we determined the B cell differentiation state by high-throughput flow cytometry. By screening 2 donors we identified 10 B cell differentiation factors that altered surface IgG, induced CD38/CD138 (plasmablasts/plasma cells), and/or CD27 (activated/memory B cells) expression. The most prominent hits included type I interferons which strongly induced CD38. A similar phenotype for type I interferons was previously reported in a different model system. Interestingly, both soluble FasL (sFasL) and MAp19 induced differentiation of B cells into plasmablasts and plasma cells. Moreover, sFASL and MAp19 increased the secretion of IgG1 and IgG4 isotypes, showing that both proteins augment the induction of functional plasma cells. Notably, FasR was upregulated on memory B cells upon CD40L-CD40 interaction, suggesting that cognate sFasL-FasR is important for the specific differentiation into plasma cells. Thus this first secretome screen for B cell differentiation identified new regulators of B cell differentiation.

P.A4.02.17

PI3K as the main driver of actin-dependent particulate antigen acquisition by human B cells

N. J. M. Verstegen[1,2], *P. A. Unger*[1], *J. Z. Walker*[1], *B. P. Nicolet*[1], *T. Jorritsma*[1], *J. van Rijssel*[3], *R. Spaapens*[1], *J. de Wit*[1], *J. D. van Buul*[3], *A. ten Brinke*[1], *M. van Ham*[1,2];
[1]*Department of Immunopathology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands,* [2]*Department of Synthetic Systems Biology and Nuclear Organization, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands,* [3]*Department of Molecular Cell Biology, Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands.*

To induce antibody production, B cells internalize foreign antigen during infection or self-particles, like cell remnants, in auto-immunity and alloimmunization after blood transfusion. This internalization requires antigen recognition by the B cell receptor (BCR) and is needed to present antigenic peptides via MHC II to attract the $CD4^+$ T cell help required for class switching, somatic hypermutation and plasma cell differentiation. Often antigen is of a particulate nature. Although well described that B cells internalize particulate antigen, the underlying molecular pathways remain undefined. Using a high-throughput quantitative image analysis approach, we demonstrate that BCR-mediated signaling to PI3K promotes actin-driven particulate antigen acquisition and $CD4^+$ T cell activation. According to current dogma, PI3K is recruited to the BCR via CD19, as part of the co-receptor. Strikingly, using the CRISPR-Cas9 technique, we demonstrate that CD19 is not required for BCR-mediated internalization of particulate antigen by human B cells. The redundancy in PI3K recruitment to facilitate particulate antigen internalization is mediated by the adaptor protein Nck. Human B cells thus employ the direct BCR-Nck-PI3K axis to modulate the actin cytoskeleton without clear CD19 co-receptor involvement for acquisition of particulate antigen. This knowledge may help to develop therapeutic agents to prevent auto- or alloantibody induction in auto-immune diseases and transfusion in which internalization of large cell fragments containing self-antigen by B cells play a central role.

P.A4.02.18

A splenic IgM memory subset harboring anti-bacterial specificities is sustained from persistent mucosal germinal center reactions.

S. WELLER*;*
INSERM U1151-CNRS UMR 8253, PARIS, France.

To what extent immune responses against the gut flora are compartmentalized within mucosal lymphoid tissues in the absence of inflammation or external aggressions, remains a much-debated issue. We describe here, based on an inducible AID fate mapping mouse model, that systemic memory B cell subsets, including mainly IgM^+ B cells, together with IgA^+ B and plasma cells in the spleen, and IgA^+ plasma cells in bone marrow, are generated in mice in the absence of deliberate immunization. While the IgA component appears dependent upon the gut flora, IgM memory B cells are still generated in germfree mice, albeit to a reduced extent and with reduced Ig gene diversification. Clonal relationships, renewal kinetics after anti-CD20 treatment, and BrdU labeling reveal that this long-lasting splenic population is to a large extent maintained by constant output of B cell clones persisting in Peyer's patch and mesenteric lymph node germinal centers.
IgM-secreting hybridomas established from splenic IgM memory B cells showed reactivity against gut luminal content, $Gram^+$ and $Gram^-$ bacterial isolates and endogenous retroviruses. Ongoing activation of B cells in gut-associated lymphoid tissues thus generates a large systemic compartment showing long-lasting clonal persistence and harboring cross-reactive antigenic specificities endowing them with a protective capacity against systemic bacterial infections. This study reveals a new layer of protection achieved by diversified IgM memory B cells generated in homeostatic conditions.

P.A4.02.19

The role of Krüppel-Like-Factor 2 (KLF2) transcription factor in plasma cell homeostasis

J. Wittner[1,2], *H. Jäck*[1,2], *W. Schuh*[1,2];
[1]*Division of Molecular Immunology, Department of Internal Medicine III, University Hospital Erlangen, FAU Erlangen/Nürnberg, Erlangen, Germany,* [2]*Transregional Collaborative Research Centre TRR130: B cells: Immunity and Autoimmunity, Erlangen, Germany.*

Krüppel-Like-Factor 2 (KLF2), as a key regulator of cell differentiation and organ development, is known to regulate quiescence, migration and homing of B cells, plasmablasts (PB) and plasma cells (PC). Upon boost immunization, KLF2-deficient mice show a striking reduction in the number of antigen-specific IgG plasma cells in the bone marrow.
To determine the effect of KLF2 on plasma cell homeostasis in more detail, we analyzed CD138+/TACI+ PB/PC subpopulations in various lymphatic organs such as spleen, bone marrow (BM) and gut associated lymphoid tissues (GALT) for their isotype expression and antibody secretion by flow cytometry and Elispot. We found that frequencies of IgM-, IgA- and IgG producing CD138+/TACI+ PBs and PCs are altered in BM and spleen of KLF2-deficient mice. The vast majority of PBs and PCs in spleen and BM of KLF2-deficient mice were IgM positive. In addition, lowered serum IgA as well as fecal (s)IgA levels indicate a crucial role of KLF2 for migration and/or survival of class switched plasma cells.
To unravel the role of KLF2 for PB/PC homeostasis, we are currently investigating KLF2-dependent target genes and signaling pathways by analyzing gene expression profiles of KLF2-deficient PBs and PCs in comparison to their wildtype counter parts. Furthermore, we are performing transfer experiments with KLF2-deficient PB/PC to analyze their migration and homing behavior.
This work was supported, in part, by the Deutsche Forschungsgemeinschaft (DFG) through research grant TRR130 (project P09).

P.A4.02.20

Receptor signals in a MyD88 L265P mutation-driven murine model of diffuse large B cell lymphoma

O. Wolz[1], *G. Knittel*[2], *Y. Cardona*[1], *F. Herster*[1], *H. Kashkar*[3], *C. Reinhardt*[2], *A. Weber*[1];
[1]*Department of Immunology, Tübingen, Germany,* [2]*University Hospital of Cologne, Cologne, Germany,* [3]*Institute for Medical Microbiology, Immunology and Hygiene, CECAD, Cologne, Germany.*

Diffuse large B cell lymphoma (DLBCL) is one of the most abundant and aggressive tumors of the hematopoietic system and remains a clinical challenge. Especially, the activated B cell (ABC) subtype of DLBCL is characterized by a high relapse rate and poor five year survival of less than 40%. 30% of ABC DLBCL tumors have a recurrent leucine 265 to proline (*L265P*) mutation in the adaptor protein MyD88, which transmits signals of Toll-like receptors (TLR), the IL-1 receptor (IL-1R) and the BAFF/APRIL-sensing receptor TACI. MyD88 L265P stimulates lymphomagenesis by constitutively activating the transcription factor NF-κB. We showed previously, that the L265P mutant strongly binds wild type MyD88, which seeds signaling complexes of a high molecular weight, the so-called Myddosomes. Consequently, blocking of MyD88 dimerization sites killed L265P-mutated DLBCL cells better than wild type controls. Whether MyD88 L265P Myddosomes form spontaneously or are triggered by beforehand mentioned upstream receptors or in combination with B cell receptor (BCR) signals is not understood. In a novel mouse model, in which B cell-specific expression of a *Myd88p.L252P* allele (murine orthologue to human *MYD88 L265P*) causes an ABC DLBCL-like disease, we have begun to analyze *in vitro* the effects of stimulating or inhibiting TLRs, IL-1R and TACI on proliferation, cytokine secretion and Myddosome signaling. This is with a view to study the impact of these receptors on lymphomagenesis *in vivo*, which may add to the treatment of the many patients carrying the somatic *MYD88 L265P* mutation.

P.A4.02.21

B cell specific interleukin 6 production plays a paradoxical role in B cell differentiation

M. AKKAYA*, S. K. Pierce;*
National Institutes of Health, Rockville, United States.

B cells secrete interleukin 6 (IL-6) in response to TLR agonists or CD40L stimulation yet the significance of B cell specific IL-6 secretion in the progression of immune response is largely unknown. Here we show on isolated mouse splenic B cells that, among different TLR agonists, TLR9 agonist CpG induces the highest magnitude of IL-6 secretion which is antagonized by B cell receptor signaling. However IL-6 KO B cells responded normally to CpG in terms of proliferation,prevention of activation induced cell death and activation marker expression *in vitro*. We then generated bone marrow chimeric mice by transferring bone marrow from B cell defficient mice mixed with bone marrow from either IL-6 KO or WT mice at 9:1 ratio and obtained B cell specific IL-6 KO mice and control mice respectively. Upon reconstitution, these mice were challenged with a T cell dependent antigen adjuvanted with either alum or TLR9 agonist CpG. We showed that B cell specific IL-6 deficiency resulted in weaker germinal center responses, lower number of antigens pecific plasma cells, lower titers of specific antibodies and less antibody affinity maturation upon immunization with alum adsorbed antigen. Paradoxically, the same responses enhanced when antigen is adjuvanted with CpG.These results suggest that, in the absence of CpG, B cell mediated IL-6 secretion is driven by B-T interactions and works in favor of T dependent responses. However, in the presence of CpG, B cell specific IL-6 production favors early commitment towards T independent plasma cell generation and thus plays role in dampening germinal center formation and affinity maturation.

P.A4.02.22

Regulation of B cell development and differentiation through Type I interferon signalling

M. AKKAYA[1], *B. Akkaya*[1], *A. Gangaplara*[2], *A. S. Roesler*[1], *B. P. Theall*[1], *S. K. Pierce*[1];
[1]*National Institutes of Health, Rockville, United States,* [2]*National Institutes of Health, Bethesda, United States.*

Type I interferons (IFNs) are among the soluble mediators of anti-viral immune response. Although innate immune cells are responsible for the majority of type I IFN production in the body, recently we showed that B cells, when stimulated with lipid conjugated CpG-A type TLR9 agonists produce Type-I IFNs. However, other pathways that can induce Type I IFN in B cells and the overall significance of B cell specific IFN production have yet to be characterized. Here, upon testing a range of stimulation conditions, we showed that dual stimulation of TLR3 and TLR4 is a potent inducer of type I IFNs in murine splenic B cells. Furthermore, by comparing WT and IFNAR KO mice, we identified that TLR induced upregulations of CD86 and CD317 on B cells are dependent on autocrine effects of IFNs. We then tested the extent to which B cell responses are mediated by IFNs by generating bone marrow chimeric mice using 1:1 ratio of WT and IFNAR KO bone marrow cells. Upon reconstitution, 1:1 ratio was conserved in total B cells and follicular cell compartments. However, majority of B1 and marginal zone cells came from WT mice while transitional cells showed IFNAR KO predominance indicating the differential effects of IFN signaling in B cell development. Once chimeric mice were infected with LCMV, we observed a significant increase in WT/IFNAR KO ratio in class switched B cells despite relatively unchanged ratios in germinal center and plasma cell compartments. These findings indicate that Type I IFNs play major roles at various stages of B cell development, activation and differentiation.

P.A4.03 Germinal centers and B cell differentiation - Part 3

P.A4.03.01

Comprehensive characterization of the human plasmablast response in dengue patients from India

C. Aggarwal[1], *K. Nayak*[1], *M. Singla*[2], *S. Gunisetty*[3], *E. Reddy*[1], *H. Panda*[1], *G. Medigeshi*[4], *R. Lodha*[2], *S. Kabra*[2], *R. Ahmed*[3], *A. Chandele*[1], *M. Kaja*[1];
[1]*International Centre for Genetic Enginnering and Biotechnology (ICGEB), New Delhi, India,* [2]*All India Institute of Medical Sciences (AIIMS), New Delhi, India,* [3]*Emory Vaccine Centre, ATLANTA, United States,* [4]*Translational Health Science and Technology Institute (THSTI), Faridabad, India.*

Humoral immune responses are thought to play a major role in dengue virus-induced immunopathology; however, little is known about the plasmablasts. Plasmablasts are terminally differentiated antibody secreting cells. The timing of their transient appearance in peripheral blood strikingly coincides with the presence of an infection /vaccination. Only a handful of studies from different parts of the world and none from India, have analyzed the B cell response in dengue infection. However, none of these studies were designed to comprehensively study the B cell response that includes phenotype and functionality.
Here, we characterize the magnitude, specificity, and kinetics of the plasmablast response in acute febrile Dengue patients from New Delhi, India. We observe a massive plasmablast response which is heterogeneous and varies from 1% to 85% of B cells and averages at 22.5%. Longitudinal analysis showed that the plasmablasts can expand dramatically with frequencies increasing by more than 10-fold within a short window of 24-48hrs. A vast majority of these plasmablast produces dengue specific IgG irrespective of whether the patient was primary or secondary infection. Also, the frequency of plasmablast was not significantly different in primary and secondary infection, but the rapidity of expansion was faster in secondary infection.
This study is the first detailed analysis of the plasmablast response in Dengue patients in India and seeks to address whether plasmablasts are associated with clinical outcome and understand the biological mechanisms that determine the balance between protection versus pathology in dengue, which is critical for devising rational prevention/ control measures.

P.A4.03.02

Impact of type-1 and type-2 adjuvants on T follicular helper, T follicular regulatory and germinal center B cell populations

A. P. Basto[1], *A. Maceiras*[1,2,3], *S. C. Almeida*[1,4], *P. Campos*[1], *F. Ribeiro*[1], *S. Kumar*[1], *M. Russo*[5], *L. Graca*[1,2];
[1]*Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisboa, Portugal,* [2]*Instituto Gulbenkian de Ciência, 2780-156 Oeiras, Portugal,* [3]*Current affiliations: i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, 4200-135 Porto, Portugal; and IBMC – Instituto de Biologia Celular e Molecular, Universidade do Porto, 4200-135 Porto, Portugal,* [4]*Current affiliation: Núcleo de Doenças Infecciosas (NDI), Universidade Federal do Espírito Santo (UFES), Vitória - ES, 29047-100, Brazil,* [5]*Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, São Paulo - SP, CEP 05508-900, Brazil.*

The generation of antibody responses is crucial for the protective immunity induced by most effective vaccines. The efficacy of those vaccines relies not only on the magnitude but also on the type, persistence and affinity of the antibodies elicited. These qualitative properties of the humoral immune response depend on appropriate germinal center (GC) reactions, which are supported by T follicular helper (Tfh) cells and regulated by T follicular regulatory (Tfr) cells. Although it is known that different adjuvants induce distinct subclasses of antibodies, their influence on GC reactions is yet poorly understood. Here we investigated the impact of a panel of type-1 and type-2 adjuvants on Tfh, Tfr and GC B cell populations. Mice were immunized with the same model antigen (ovalbumin) in combination with Th2-polarizing adjuvants (potassium alum or incomplete Freund's adjuvant) or with three different formulations containing a toll-like receptor (TLR)9 ligand with Th1-polarizing properties (CpG oligodeoxynucleotides). As expected, Th2-type adjuvants induced an IgG1 class-switch whether formulations containing the TLR9 ligand induced higher IgG2a responses. We found that Th2-adjuvants were more effective in inducing proliferation of GC B cells ($CD19^+Fas^+GL7^+$) but this effect could not be ascribed to quantitative differences in Tfh or Tfr populations, since $CD4^+CXCR5^+PD1^+$ cell levels and Tfh:Tfr ratios did not correlate with GC B cell expansion. Similar conclusions were obtained from Th1-prone (C57BL/6) and Th2-prone (Balb/c) mouse strains. Qualitative differences of Tfh populations generated under Th1- versus Th2-polarizing conditions are now being evaluated through differential gene expression analysis.

P.A4.03.03

Phenotypical and functional characterization of IL-10-producing regulatory B cell subsets

V. Duong, *N. Farimany, N. Föger, K. Lee;*
Inflammation Research Group,Institute of Clinical Chemistry,Hannover Medical School, Hannover, Germany.

Recent studies have recognized that B cells can negatively regulate pathogenic T cell responses in an antibody-independent manner, which has led to the concept of regulatory B cells. Regulatory B cells have been functionally defined in mice and humans by their ability to produce the anti-inflammatory cytokine IL-10 and have been suggested to exhibit immunosuppressive function in the context of autoimmune and inflammatory disease. However, the nature and origin of regulatory B cells is controversial and it is still unclear whether they represent a distinct B cell lineage or a dynamic cellular state. Our study reveals that regulatory B cells are not a predetermined novel B cell lineage, but are formed by a pool of innate-like 'classical' B cells. Our data show that the majority of IL-10-competent regulatory B cell subsets resides within both B1 and B2 B cell compartments and originates from B1a and marginal zone B cells. We directly demonstrate that these IL-10-producing B cell subsets exhibit suppressive function on the production of proinflammatory cytokines by $CD4^+$ and $CD8^+$ T cells. Moreover, these regulatory B cells exhibit a high degree of self-reactivity and produce autoreactive antibodies directed against dsDNA and ssDNA. Our study further suggests that regulatory B cell differentiation is guided by BCR-responsiveness and signaling thresholds. Thus, regulatory B cells act as an immunological double-edged sword: they exhibit potent immunoregulatory activity on inflammatory T cell responses, but, owing to a high degree of self-reactivity, can also turn into harmful autoantibody-producing plasma cells.

P.A4.03.04

Dissecting the T cell-extrinsic requirements for Tfh cell differentiation

J. Huber, *D. Baumjohann;*
Institute for Immunology, Planegg-Martinsried, Germany.

T follicular helper (Tfh) cells represent the primary CD4+ T cell subset that provides crucial help to B cells for potent antibody responses during infection and vaccination. While there is emerging evidence for the molecular mechanisms in T cells that drive Tfh cell differentiation, e.g. up-regulation of the Tfh cell-associated transcription factor Bcl6 and down-regulation of the Bcl6 antagonist Blimp-1, the T cell-extrinsic signals that induce the Tfh cell fate remain largely unknown. Dendritic cells (DCs) are potent inducers of the Tfh cell phenotype and this induction is independent of cognate interactions with B cells. Thus, DC-derived signals seem to instruct molecular programs in responding T cells. While a differential role of splenic CD8+ and CD8– DC subsets has been reported for the activation of CD8+ or CD4+ T cells, respectively, less is known about the DC subset(s) that induce(s) Tfh cells and which transcription factor networks and co-stimulatory pathways are involved in the DC-T cell interactions that drive Th cell fate decisions. Here, we tested how the Bcl6/Blimp-1 axis in DCs impacted their ability to induce Tfh cells. These data provide new insights into the mechanisms by which DCs induce Tfh cells and shape their identity.

P.A4.03.05

Contribution of amino acid micro-environment to the glycosylation status of N-glycosylation sites in antibody VH4 genes

***J. Koers**, N. Derksen, P. Ooijevaar-de Heer, T. Rispens;*
Sanquin Research, Amsterdam, Netherlands.

Approximately 15% of IgG antibodies acquire glycans in the Fab domain on consensus amino acid motifs (Asn-X-Thr/Ser, X ≠ Pro), so-called *N*-linked glycosylation sites. Once acquired, Fab glycans can affect antibody stability and affinity. The human naïve B cell repertoire is largely devoid of *N*-linked glycosylation sites with only a few germline-encoded alleles displaying such sites. IGHV4-34 is such an allele and besides having an germline-encoded *N*-linked glycosylation site (N_H57) it is also one of the more frequently used human V_H alleles. However, despite being a canonical N-glycosylation site, it usually remains non-glycosylated, most likely due to the surrounding amino acid micro-environment. It has been shown that charge and hydrophobicity of the core amino acid X of the consensus motif plays an important role in likelihood of glycosylation. Interestingly, the germline N_H57 core amino acid X is histidine which was shown to be favorable for glycosylation. This raises the question whether neighboring amino acids can also influence glycosylation status of the consensus motif. Furthermore, other VH4 family members have the potential to gain this site in the CDR2 upon somatic hypermutation. By introducing the N_H57 glycosylation site in multiple IGHV4 IgG clones from several VH4 family genes we can investigate its glycosylation status in the context of different neighboring amino acids. Thereby, we intend to demonstrate the contribution of adjacent and core amino acids in the glycosylation status of the canonical or potential SHM induced N_H57 glycosylation site in VH4 gene family members.

P.A4.03.06

Modeling transcriptomics of T follicular helper cells across different strains of mice.

***S. Kumar**[1], S. C. Almeida[2], P. Campos[1], A. Bastos[1], M. Russo[3], M. Carvalho[4], L. Graça[1];*
[1]Instituto de Medicina Molecular, Lisbon, Portugal, [2]Núcleo de Doenças Infecciosas (NDI), Universidade Federal do Espírito Santo (UFES), Vitoria, Brazil, [3]Instituto de Ciências Biomédicas,Departamento de Imunologia, Universidade de São Paulo, São Paulo, Brazil, [4]BioISI - Institute for Biosystems and Integrative Sciences, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal.

It has been known that different inbred mouse strains show highly varied immune responses as a consequence of mutations and polymorphisms. However, the impact of these variations leading to differing immune response is not only limited at genetic level. Previous studies have shown that transcriptome variance in different mouse strains also has significant impact on the phenotype of immune cells and as such it is essential to investigate how transcriptomic variations from different mouse strains impact the immune responses. In T-cell dependent immune responses, T follicular helper cells play an important role in antibody production. These cells drive germinal centre reactions by interacting with B cells and provide signals required for somatic hypermutation, affinity maturation, and thus the development of high affinity antibodies. In this project, we model the transcriptomics of T follicular helper (Tfh) cells in two different strains of mice. We have generated RNA-seq data from C57BL/6J and Balb/c.ByJ immunized against same antigen using type 1 and type 2 adjuvants. These two mouse strains are well known for their stronger preference towards type 1 and type 2 responses respectively. Here we discuss some results of transcriptomics differences in Tfh cells across BALB/c and B6 mice.

P.A4.03.07

The deciphering of phenotypic and functional B cell defects in IgA deficiency

***A. L. Lemarquis**[1,2], F. P. Theodors[1], S. H. Lund[3], I. Jonsdottir[1], H. K. Einarsdottir[1], B. R. Ludviksson[1];*
[1]University Hospital of Iceland, Reykjavík, Iceland, [2]Faculty of Medicine, University of Iceland, Reykjavík, Iceland, Iceland, [3]University of Iceland, Reykjavík, Iceland.

Selective IgA deficiency (IgAD) is the most common primary antibody deficiency in Caucasians with affected individuals suffering from an increased burden of autoimmunity with autoantibody positivity, atopy and infections. Our *ex vivo* analysis of lymphocyte populations in IgAD shows B cell defects with significantly lower numbers of transitional B cells ($CD19^+CD24^{hi}CD38^{hi}$) and class-switched memory B cells ($CD20^+CD27^+IgD^-$). A novel T cell independent defect was seen *in vitro* after CpG (ODN-2006) stimulation where it failed to induce IgA production and enhanced the defect in transitional B cells, especially within B regulatory cells expressing IL-10. Proportions of T cell populations *ex vivo* as well as *in vitro* induced T effector cells and T regulatory cells remained however normal. The signalling analysis of ERK was normal after CpG while pSTAT3 showed decreased signal in both total T and B cells following IL-21 stimulation. In serum from IgAD individuals a raised concentration of sCD40L was seen without stimulation, correlating inversely with IgA in IgAD but not IgG and IgM.
Pathway enrichment analysis of mRNA transcriptomics of isolated B cells from IgAD individuals before and after CpG stimulation points towards defects related to longevity and survival. Collectively, our data indicates a complex B cell defect with defects in both T cell dependent and independent responses and overproduction of CD40L pointing towards a compensatory B cell overstimulation which may be important to assess in the treatment with “personalised immunotherapies” of individuals affected by IgAD and concomitant immunodysregulation.

P.A4.03.08

The mutation patterns in B-cell immunoglobulin receptors reflect the influence of selection acting at multiple time-scales

***Y. Louzoun**[1], G. Yaari[1], s. kleinstein[2];*
[1]Bar Ilan, Ramat Gan, Israel, [2]Yale, New Haven, United States.

During the adaptive immune response, B cells undergo a processof clonal expansion, somatic hypermutation of the immunoglobulin (Ig) genes and affinity-dependent selection. Over a lifetime, each B cell may participate in multiple rounds of affinity maturation as part of different immune responses. These two time-scales for selection are apparent in the structure of B-cell lineage trees, which often contain a ‘trunk’ consisting of mutations that are shared across all members of a clone, and several branches that form a ‘canopy’ consisting of mutations that are shared by a subset of clone members. The influence of affinity maturation on the B-cell population can be inferred through the pattern of somatic mutations in the Ig. While global analysis of mutation patterns has shown evidence of strong selection pressures shaping the B-cell population, the effect of different time-scales of selection and diversification has not yet been studied. Analysis of B cells from blood samples of three healthy individuals identifies a range of clone sizes with lineage trees that can contain long trunks and canopies. We here show that observed mutation patterns in the framework regions (FWRs) are determined by an almost purely purifying selection on both short and long time-scales. By contrast, complementarity determining regions (CDRs) are affected by a combination of purifying and antigen-driven positive selection on the short term, which leads to a net positive selection in the long term. In both the FWRs and CDRs, long-term selection is strongly dependent on the heavy chain variable gene family.

P.A4.03.09

Converging Evolution Leads to Near Maximal junction diversity through parallel mechanisms in B and T cell receptors

***Y. Louzoun**, J. Benichou;*
Bar Ilan, Ramat Gan, Israel.

T and B cell receptor (TCR and BCR) complementarity determining region 3 (CDR3) genetic diversity is produced through multiple diversification and selection stages. Potential holes in the CDR3 repertoire were argued to be linked to immunodeficiencies and diseases. In contrast with BCRs, TCRs have practically no Dβ germline genetic diversity, and the question whether they can produce a diverse CDR3 repertoire emerges.
In order to address the genetic diversity of the adaptive immune system, appropriate quantitative measures for diversity and large scale sequencing are required. Such diversity method should incorporate the complex diversification mechanisms of the adaptive immune response and the BCR and TCR loci structure.
We combined large-scale sequencing and diversity measures to show that TCRs have a near maximal CDR3 genetic diversity. Specifically, TCR have a larger junctional and V germline diversity, which starts more 5’ in Vβ than BCRs. Selection decreases the TCR repertoire diversity, but does not affect BCR repertoire. As a result, TCR is as diverse as BCR repertoire, with a biased CDR3 length toward short TCRs and long BCRs. These differences suggest parallel converging evolutionary tracks to reach the required diversity to avoid holes in the CDR3 repertoire.

P.A4.03.10

Inflammation mediated by T-bet during blood-stage infection modulates the development of humoral immunity to malaria

***A. Ly**[1,2], L. Yang[1,2], W. Shi[1,2], V. Ryg-Cornejo[1,2], L. Ioannidis[1,2], K. L. Jacobson[3], G. T. Belz[1,2], A. Kallies[1,4,5], D. S. Hansen[1,2];*
[1]The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia, [2]The University of Melbourne, Department of Medical Biology, Parkville, Australia, [3]Monash University, Department of Biochemistry and Molecular Biology, Clayton, Australia, [4]The University of Melbourne, Peter Doherty Institute for Infection and Immunity, Parkville, Australia, [5]The University of Melbourne, Department of Microbiology and Immunology, Parkville, Australia.

Malaria is a preventable but serious infectious disease that burdens developing countries, in part because natural immunity to the malaria-causing *Plasmodium* parasites takes years of repeated infections to develop. The reasons for this are elusive; however, growing immuno-epidemiological evidence indicate that parasite-specific antibodies and memory B cells (MBCs) that mediate protection, are poorly generated and short-lived in endemic areas. In contrast, MBCs can be induced effectively in low transmission settings, suggesting that acute infection may undermine the acquisition of humoral immunity. Using a preclinical model of malaria, we demonstrated that infection-associated pro-inflammatory pathways hinder germinal centre (GC) and MBC development. To delineate the underlying molecular mechanisms, GC responses to blood-stage *P. berghei* ANKA was examined in mice deficient in the pro-inflammatory, T helper 1 transcription factor, T-bet. Genetic ablation of T-bet in CD4 T cells significantly improved the differentiation rates of T follicular helper cells, consequently boosting GC and antibody responses to infection.

T-bet not only affected T cell help, but also modulated intrinsic B cell activity. Deletion of T-bet in B cells enhanced the GC response to infection, and altered its dark and light zone dynamics, critical for high-affinity antibody production. RNA sequencing further revealed that T-bet significantly modulated the GC B cell transcriptional program during infection. We show that inflammation driven by T-bet during blood-stage malaria suppresses the acquisition of humoral immunity, by compromising T cell help and GC B cell differentiation. This knowledge will inform next generation vaccine and therapeutic strategies to elicit sustained protection against disease.

P.A4.03.12
Human Antibody Transgenic Rabbits

S. Offner, *F. Ros, E. Koenigsberger, S. Klostermann, H. Niersbach;*
Roche Diagnostics GmbH, Penzberg, Germany.

Antibodies generated in animal hosts make up the larger part of marketed therapeutic antibodies. Their generation undergoes positive and negative selection as well as affinity maturation in vivo, which lead to antibodies with robust therapeutic properties. Rabbits use a distinct diversity generation mechanism, which has been exploited since long to achieve very high specificity and affinity. However, antibodies derived from wild type animals such as mouse, hamster, rat or rabbit still have to undergo a process called "humanization" before being used as therapeutics in humans, limiting flexibility and adding time to the drug development process. Here, we report the development of rabbits transgenic for human immunoglobulin genes and the characterization of their B-cell compartment. Highly affine antibody candidates were yielded from these human antibody transgenic rabbits showing an excellent "humanness" and a specific therapeutic mode of action.

P.A4.03.13
Role of dendritic cells in antigen transport, transfer and B cell activation

M. Capitao, S. Borreill, H. El-barbry, F. Niedergang, ***F. OUAAZ****;*
Institut Cochin-INSERM U1016, Paris, France.

Dendritic cells (DCs) are phagocytic cells, which sample antigen (Ag) in the periphery and migrate to the lymph node (LN) where they activate T cells and potentially B cells. Previously, we reported that DCs can transfer Ag to B cells after an extracellular release that was called "regurgitation". The modes of B cell activation by DCs and the underlying mechanisms still remain unknown. We have investigated: 1)- Ag transport by DCs and distribution in the LN, 2)- the role of DCs in B-cell activation *in vivo* and in co-culture *in vitro*, 3)- the mechanisms of early B cell activation by analysing NF-κB pathway in B cells. Ag-carrying DCs migrated to LN within 18h after footpad mice injection allowing to Ag colocalization with both $CD11b^+$ and $CD8\alpha^+$ resident DCs. At 48h, we observed proximal positionning of Ag with follicular B cells. At 72h, Ag-loaded DCs were able to induce B cell activation and differentiation *in vivo*. In co-culture *in vitro*, Ag-loaded DCs activated anti-HEL B cells and importantly Ag release in DC supernatant by regurgitation induced also early B cell activation. BCR stimulation with soluble Ag and Ag released by DCs were able to activate NF-κB in particular cRel subunit nuclear mobilization. DCs are an important cell transporter of native Ag from the periphery and also activators of B cells *in vivo* and in co-cultures *in vitro.* We expect to provide new insights into Ag encounter by B cells *in vivo*, and novel approaches of DC targeting to elicit humoral immunity.

P.A4.03.14
CLASS SWITCH RECOMBINATION DEFICIENCIES MOROCCAN CASES SERIES

H. OUAIR*[1,2], I. Benhsaien[3], J. El Bakkouri[3], L. Jeddane[4], H. Salih Alj[5], A. Bousfiha[6], F. Ailal[4];*
[1]Biology Laboratory and Health/ Immune and Metabolic Pathology Team- Faculty of Sciences Ben M'Sik. Hassan II University, casablanca, Morocco, [2]University Hospital Casablanca-Children Hospital Abderrahim Harouchi, Casablanca, Morocco, [3]2- Laboratory of Clinical Immunology, Allergy and Inflammation- Faculty of Medicine and Pharmacy Hassan II University, casablanca, Morocco, [4]Laboratory of Clinical Immunology, Allergy and Inflammation- Faculty of Medicine and Pharmacy Hassan II University, casablanca, Morocco, [5]1- Biology Laboratory and Health/ Immune and Metabolic Pathology Team- Faculty of Sciences Ben M'Sik. Hassan II University, casablanca, Morocco, [6]Laboratory of Clinical Immunology, Allergy and Inflammation- Faculty of Medicine and Pharmacy Hassan II University, Casablanca, Morocco.

Class Switch Recombination Deficiencies (CSR-D) is characterized by a decrease serum IgA and IgG with normal or increased IgM. Several genetic mutations were defined that affects the interaction between T and B cells required to produce Ig A and G. There are two main forms: X-linked and autosomal recessive. Clinical manifestations are dominated by recurrent infections (pulmonary, ENT, digestive ...), especially with opportunistic pathogens. Our study reports 17 cases of hyper IgM followed in our department since 1995. There are 8 boys and 9 girls with 12 patients reporting a parental consanguinity. The mean age at diagnosis is 4.5 years (5 months to 12 years). The clinical manifestations were dominated by respiratory infections (13 cases), including 6 cases of bronchiectasia and two CMV pneumonia. Chronic diarrhea was observed in 4 patients with cryptosporidiosis. ENT infections were noted in 6 cases. One patient had three episodes of meningitis and another one BCGitis. All patients had low IgG and IgA and 11 patients had high levels of IgM. Five patients had thrombocytopenia.
Unlike Europe, where X-linked hyper-IgM syndrome (HIGM1) is the most common, this Moroccan series shows the frequency of autosomal recessive forms in our context as suggested by the inbreeding rate, the frequency of female and the predominance of phenotypes with lymphoproliferation.

P.A4.03.15
Transcriptional dysregulation of CVID patients harboring C104R *TNFRSF13B* mutation

N. J. Ramirez*[1,2,3], N. Langer[1], C. Bossen[1], B. Grimbacher[1];*
[1]Centre for Chronic Immunodeficiency (CCI), University Medical Centre Freiburg, Freiburg, Germany, [2]Integrated Research Training Group (IRTG) Medical Epigenetics, Collaborative Research Centre 992, Freiburg, Germany, [3]Faculty of Biology, Albert-Ludwigs-University of Freiburg, Freiburg, Germany.

Objective- To determine additional factors contributing to the development of common variable immunodeficiency (CVID), we investigated the perturbations of the transcription factor binding in CVID patients harboring the C104R *TNFRSF13B* mutation, encoding the protein TACI, compared to their healthy relatives harboring the same *TNFRSF13B* mutation and healthy donors tested negative for the mutation. **Methods-** Assay for transposase accessible chromatin-sequencing (ATAC-seq) was performed on naïve and class switched memory B cells of three heterozygous CVID patients, three healthy relatives carrying the same heterozygous C104R *TNFRSF13B* mutation, three homozygous CVID patients and four healthy donors, to determine open regions of chromatin. In addition, ATAC-seq was performed on $CD14^+$ monocytes and $CD4^+$ naïve T cells of two of the heterozygous CVID patients and their healthy controls. **Results-** Our analysis revealed 14 467 regions differentially regulated in B cell subtypes between naïve and class-switched memory. Comparison of TACI mutant carriers and healthy donors identified 1001 regions preferentially open in TACI mutant carriers with the most enriched TFBM for NF-κB (p-value 1×10^{-239}). Comparison of monocytes and T cell subpopulations from CVID patients and their healthy controls showed no NF-κB dysregulation. **Conclusion-** Here we could show transcriptional dysregulation in naïve and class-switched memory B cells of TACI C104R carriers compared to healthy individuals with the most enriched TFBM for NF-κB, which is essential for regulating immune response to infections. Thus, its changes can lead to variety of disease-causing differences. Furthermore, the data suggest NF-κB dysregulation is B cell intrinsic.

P.A4.03.16
Sequence representation in Germinal Centre simulations

P. A. Robert*[1], M. Meyer-Hermann[2];*
[1]Helmholtz Zentrum für Infektionsforschung, Braunschweig, Germany, [2]Helmholtz Zentrum für Infektionsforschung and Institute for Biochemistry, Biotechnology and Bioinformatics, Technische Universität Braunschweig, Braunschweig, Germany.

The process of affinity maturation in Germinal Centres is critical for the production of efficient antibodies and recall responses against pathogens. The dynamics of B cell movement, proliferation, selection and thus affinity maturation inside Germinal Centres can be successfully captured by mathematical modelling. These models rely on an abstract representation of protein-protein interactions to translate the effects of somatic hypermutation into a change in affinity to the antigen. In light of recent challenges for the development of broadly neutralizing vaccines, it would be of interest to study the effect of multiple antigens or epitopes driving Germinal Centre dynamics. The sequence representation used in silico so far has structural biases and potential effects onto the emerging cross-reactivity of receptors produced by the Germinal Centres. Here, we compare the consequences of different representations of sequences onto the properties of affinity maturation. The results help to understand the efficiency of different vaccination regimens, and to solve emerging contradictions regarding the vaccination strategies using cocktails of antigens at the same time or following sequential immunizations.

P.A4.03.17
The immunoglobulin tail tyrosine motif in transmembrane IgE is required for generation and/or survival of IgE-producing plasma cells

M. E. R. Schmitt*[1], N. Engels[2], J. Wienands[2], D. Vöhringer[1];*
[1]Department of Infection Biology, Institute for Clinical Microbiology, Immunology and Hygiene, University Hospital and Friedrich-Alexander University (FAU), Erlangen-Nürnberg, Germany, [2]Institute of Cellular and Molecular Immunology, Georg August University, Göttingen, Germany.

Introduction: Immunoglobulins of the IgE isotype are associated with allergic inflammation and immunity against helminths. Affinity-matured IgE-producing plasma cells (PCs) are thought to be mainly generated by sequential class switch recombination via an IgG1 intermediate step. However, the generation and homeostasis of IgE-producing plasma cells (PC) is still poorly understood.

Objectives and Methods: To investigate whether the cytoplasmic tail of mIgG1 or mIgE is important for the IgE response we analysed mutant mice after infection with the helminth *Nippostrongylus brasiliensis.* We compared IgG1YF and IgEYF mice in which the immunoglobulin tail tyrosine (ITT) motif in mIgG1 or mIgE is inactive. In addition we used IgEKVK mice with a truncated cytoplasmic IgE tail.
Results: Our results show a normal IgE memory response in IgG1YF mice. In contrast, IgE+ PCs in IgEYF mice were reduced after secondary infection. In IgEKVK mice the IgE+ PCs were diminished after first and second infection. Serum IgE levels were reduced in both IgE mutant mice.
Conclusion: Our results show that the ITT motif in mIgG1 is important for the IgG1, but not the IgE memory response. However the ITT motif in mIgE and the intracellular part of IgE are important for the IgE response. This led to the hypothesis that the ITT motif in mIgE is important for the generation or survival of IgE+ PCs and we will now investigate whether this hypothesis can be validated.

P.A4.03.18
Hyperimmunization with RhD generates a broad and persistent repertoire of IgM$^-$ and IgG$^-$ antigen-specific B cells

*M. Berkowska[1], **N. van der Bolt**[1], L. Della Valle[1], H. IJspeert[2], O. Verhagen[1], M. van der Burg[2], A. ten Brinke[1], M. van Ham[1], G. Vidarsson[1], E. van der Schoo[1];*
[1]Sanquin Research, Amsterdam, Netherlands, [2]Leiden UMC, Leiden, Netherlands.

RhD-negative women, pregnant with a RhD-positive child, receive anti-D immunoglobulins (Ig) from hyperimmunized donors to prevent alloimmunization. The characteristics of anti-D-Igs and evolution of the anti-D response remain poorly characterized.
RhD-specific CD27$^-$IgM$^+$, CD27$^+$IgM$^+$, CD27$^+$IgG$^+$ and CD27$^-$IgG$^+$ B cells from six anti-D donors were single cell sorted. Clones producing anti-D-Igs were subjected to Ig gene analysis to study clonal relationships. To investigate evolution of the anti-D response, known anti-D rearrangements from 3 donors from 3 time points (spanning up to 10 years) were identified using next-generation sequencing.
RhD-specific B cells, equally distributed between IgM$^+$ and IgG$^+$, were identified in all donors. Rearranged Ig genes in RhD-specific B cells carried somatic hypermutations, displayed long IgH-CDR3s and showed a broad repertoire skewed towards *IGHV4-34*, *IGHV3-33* and *IGHV3-30* genes. Frequently utilized *IGHV4-34* genes showed limited selection for replacement mutations in IgH-CDRs, implicating structural advantage in RhD recognition. ≥ 30% of identified anti-D rearrangements represented clonally related cells. The majority of clones spanned either the IgM$^+$ or IgG$^+$ B-cell subsets and clones can have life-spans of at least 10 years. Remarkably, recent time point B cells harbored less mutations then their clonally related B cells at earlier time points and the somatic hypermutations rate does not increase over time
In conclusion, the RhD response involves a broad, but restricted Ig gene repertoire, persisting over time. IgM$^+$ and IgG$^+$ B cells are important for the maintenance of a lasting alloimmune response and recruitment of IgM$^+$ memory B cells plays an important role in the memory response.

P.A4.03.19
Identification of follicular dendritic cells (FDCs) from human tonsils and binding of model complement complexes

*L. Rapley, E. Lekova, **M. SAISANA**, K. Nistala, C. Ellson;*
Glaxosmithkline R&D, Stevenage, United Kingdom.

Follicular dendritic cells (FDCs) are a cell type found in the germinal centre. Complement-opsonised antigen can traffic to the germinal centre where antigen is then colocalised with FDCs. FDCs are the only known cell type to retain whole native antigen. This intact antigen is re-presented on the FDC cell surface for periodic interaction with B cells, aiding antibody affinity maturation. This complement-binding function of FDCs has relevance to numerous diseases, including autoimmunity and HIV infection.
Published work using primary human FDCs is limited as they are low in frequency and difficult to isolate. Here we describe a method we have developed to isolate FDCs from human tonsil, obtaining 1000-3000 FDCs per donor. We have confirmed by flow cytometry, cell sorting and imaging of cell surface markers that these cells are indeed FDCs. We have found that these FDCs express the complement receptor 1 (CD35) and a subset also express complement receptor 2 (CD21). Through the antigen opsonisation process, C3 fragments are cleaved to leave C3dg which can bind to CD21 with high affinity. We have built a fluorescent model antigen incorporating C3dg and have generated robust data demonstrating binding of these complexes to FDCs.
The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol.

P.A4.03.20
microRNA-155 is essential for the proliferation and survival of plasmablast B-cells

***R. Leyland**[1,2], G. Arbore[2], L. Biggins[2], T. Henley[2], S. Andrews[2], R. Brink[3], E. Vigorito[2], M. Turner[2];*
[1]Sheffield Hallam University, Sheffield, United Kingdom, [2]Babraham Institute, Cambridge, United Kingdom, [3]Garvan Institute of Medical Research, Sydney, Australia.

The differentiation of B cells after antigen exposure is essential for antibody production and clearance of invading pathogens. During the early B-cell response to T-cell dependent antigens, B-cells differentiate into B-cell blasts, plasmablasts and germinal centre cells. Plasmablast B-cells secrete unmutated low affinity antibodies and typically differentiate into short-lived plasma cells. Although this response is short lived, it can be critical for neutralisation of rapidly dividing pathogens. The posttranscriptional regulator microRNA-155 (miR-155) has been shown to be critical for the germinal centre response; however, the cellular and molecular mechanisms by which miR-155 regulates the plasmablast response are not well understood. We utilized SW_{HEL} mice, either sufficient or deficient for miR-155, to assess the contribution of miR-155 to the plasmablast response after immunisation with hen egg lysozyme (HEL) conjugated to sheep red blood cells (HEL-SRBCs). We show that miR-155 is required to sustain the extrafollicular plasmablast response and is essential for plasmablast survival and proliferation. MiR-155 deficient, HEL-specific plasmablast B-cells showed an increase in apoptosis and defects in cell cycle progression and DNA replication compared to wild type controls. Through transcriptome analysis of miR-155 sufficient and deficient SW_{HEL} B cells we determined that miR-155 indirectly regulates genes involved in cellular processes such as the DNA metabolic process, DNA nucleosome assembly, DNA replication initiation and the mitotic cell cycle process. Overall, our data demonstrate a complex mechanism of plasmablast regulation by a single microRNA, which provides new insight into antibody production during the early response to infection and vaccination.

P.A4.03.22
B cell development sans BCR responsiveness due to unfolded protein response-triggered Mef2c protein degradation

***Y. Chen**[1], Y. Su[2], J. T. Kung[1];*
[1]Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, [2]National Laboratory Animal Center, National Applied Research Laboratories, Taipei, Taiwan.

BCR engagement leads to activation and clonal expansion of B cells. The I-$A^{12\%}$ mutant mouse possesses a branch site point mutation in the *H2-Aa* gene that causes highly reduced I-Aa protein expression. As I-A is a heterodimer made up of I-Aa and I-Ab, reduced I-Aa results not in reduced surface I-A expression but also in an excess of upaired I-Ab. B cells that develop in I-$A^{12\%}$ mice proliferated in response to LPS stimulation but failed to do so upon BCR stimulation. Developing I-$A^{12\%}$ B cells were engaged in unfolded protein response (UPR) due to an excess of unpaired I-Ab. BCR responsiveness was restored by transduced I-Aa expression and by BiP, the UPR sensor. Reducing the load of unpaired I-Ab also restored BCR responsiveness of I-$A^{12\%}$ B cells. Mef2c protein, a transcription factor required for BCR-stimulated proliferation, was missing in I-$A^{12\%}$ B cells and that transduced Mef2c expression restored BCR responsiveness. Mef2c protein appeared in I-$A^{12\%}$ B cells after addition of proteosome inhibitors. Mef2c degradation was mediated by Skp2 E3 ligase and that knock-down of Skp2 mRNA in I-$A^{12\%}$ B cells restored BCR responsiveness. Our results point to a generalized incompatibility between BCR responsiveness and increased Skp2 stability. They also imply the existence of regulatory mechanisms other than *Ig* gene rearrangement that govern Mef2c turnover in a specific, exquisite, and dynamic fashion.

P.A5.01 Initiation of immune responses - Part 1

P.A5.01.01
XCR1$^+$ Dendritic Cells are required for intestinal Th1 homeostasis

***F. Ahmadi**[1], K. Müller Luda[1], B. Malissen[2], W. W Agace[1,3];*
[1]Biomedical centre, Immunology section, Lund, Sweden, [2]Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université, INSERM, CNRS UMR,, Marseille, France, [3]Division of Immunology and Vaccinology, National Veterinary Institute, Technical University of Denmark (DTU), Kongens Lyngby, Denmark.

The intestinal mucosa contains several classical dendritic cell (cDC) subsets each of which appear to play a non-redundant role in intestinal T cell homeostasis. Intestinal cDC1, similar to cDC1 in other tissues, are dependent on the transcription factor IRF8 for their development. Utilizing CD11c-cre.IRF8$^{fl/fl}$ mice, we have recently provided data to suggest that intestinal cDC1 are important for intestinal Th1 homeostasis and priming (**1**). CD11c is however expressed by multiple cell types, including intestinal macrophages, plasmacytoid DC and subsets of B and T cells. To determine whether intestinal Th1 responses are dependent on cDC1 we have generated XCR1-Cre.IRF8$^{fl/fl}$ mice, that specifically and selectively lack cDC1. Similar to CD11c-Cre.IRF8$^{fl/fl}$ mice, XCR1-Cre.IRF8$^{fl/fl}$ mice lack Th1 cells in the small intestinal LP and displayed markedly reduced numbers of Th1 cells in the colon. In preliminary experiments, we have observed that intestinal Th1 numbers are normal in IL-12p35 or IFN-α receptor (IFNAR) deficient mice indicating that cDC1 driven intestinal Th1 homeostasis is independent of IL-12 and interferon signalling. Current studies are thus focused on identifying the key cDC1 derived factors required for intestinal Th1 homeostasis as well as the environmental triggers that promote Th1 development in the intestine.
1. Luda et al., IRF8 Transcription-Factor-Dependent Classical Dendritic Cells Are Essential for Intestinal T Cell Homeostasis, 2016, Immunity 44, 860-874.

P.A5.01.02
Toll-like receptor-dependent activation of dendritic cells in cattle

G. T. Barut[1,2,3], *S. C. Talker*[1,2], *A. Summerfield*[1,2];
[1]*Institute of Virology and Immunology, Bern and Mittelhäusern, Switzerland,* [2]*Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [3]*Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland.*

Introduction: Signaling via toll-like receptors (TLR) mediates a potent activation and maturation of dendritic cells (DC). Therefore, knowledge on TLR ligand responsiveness, which differs both with DC subset and species, is of major interest for vaccine adjuvant development. Method: Two methods to determine bovine DC activation after stimulation of PBMC with TLR ligands Gardiquimod, LPS, and Pam2CSK4 were compared: 1) Upregulation of the maturation marker chemokine receptor 7 (CCR7) as determined by flow cytometry after 4h stimulation, and 2) detection of phosphorylated p38 mitogen-activated protein kinase (MAPK) by phosphoflow after 15min. Results: After 4h stimulation of PBMC, all DC subsets upregulated CCR7 irrespective of the ligand used, which contradicted DC-subset specific TLR expression in cattle. By contrast, early detection of phosphorylated p38 in response to TLR ligands correlated with TLR gene expression data. In plasmacytoid DC, only Gardiquimod triggered p38 phosphorylation. As expected from TLR2 expression data, both conventional DC subsets were activated by Pam2CSK4. In contrast, none of the DC subsets, only monocytes responded to LPS, with increased p38 phosphorylation, again corresponding to the low TLR4 expression on blood DC of cattle. Conclusions: Bystander effects in PBMC cultures have a strong impact on TLR ligand responsiveness of DC subsets when CCR7 is analyzed after 4h. In contrast, flow cytometric detection of p38 phosphorylation in PBMC is a sensitive method to screen for functional and subset-specific TLR expression. This project was supported by the European Union's Horizon 2020 Program under Grant Agreement 633184.

P.A5.01.03
The impact of vaccine-induced innate signals on breadth and function of CD8 T cell responses

C. A. Bernhard[1], *H. Lauterbach*[2], *T. Brocker*[1];
[1]*BioMedical Center (BMC), Institute for Immunology, Planegg-Martinsried, Germany,* [2]*Bavarian Nordic GmbH, Planegg-Martinsried, Germany.*

Dendritic cells (DCs) are considered the most potent antigen-presenting cells (APCs), which directly prime or cross-prime MHC class I-restricted cytotoxic T lymphocytes (CTLs). In the context of vaccination with recombinant adenoviral vectors (rAd), we could previously demonstrate that also vaccine-infected CD169$^+$ and SIGN-R1$^+$ macrophages were sufficient to prime broad CTL responses by direct presentation in the absence of DCs (Bernhard et al., 2015).
In many cases, immunodominant CD8 T cell responses against prevalent epitopes are sufficient to mount effective anti-pathogen effector and memory CTL responses. However, for pathogens or tumors with effective immune evasion, escape from most CD8 T cell responses occurs. Then it is of great advantage to have a broad CTL repertoire for protection. However, the factors determining the capacities of vaccines to induce broad CTL responses are incompletely understood. Side-by-side comparison of CTL responses initiated by different APC types and direct comparison of vaccine vectors such as rAd and modified vaccinia virus Ankara (MVA) revealed differential potencies to induce broad vs. narrow CTL responses. Here, we analyse vaccine-intrinsic properties as well as their effects on lymphatic organs and cross-presenting XCR1$^+$ DCs that may determine the strength, breadth and quality of CTL responses after vaccination.

P.A5.01.04
In vivo effect of environmental pollution on the expression of CD64, CD14, INFgR, CD206 and AhR receptors in human alveolar macrophages infected with *Mycobacterium tuberculosis*

C. Carranza*, M. Pérez-Guzmán, M. Torres;*
National Institute of Respiratory Diseases, Mexico City, Mexico.

Introduction: The effects of air pollution on human health are a matter of great concern as more than two million deaths have been estimated to occur each year worldwide from direct damage to the respiratory system. Alveolar macrophages (AM) play an important role in the elimination of *Mycobacterium tuberculosis* (*Mtb*) and are efficient in ingesting contaminating particulate matter (PM) that have penetrated the barriers of innate immunity. The load, composition and size of PM could determine the innate and adaptive immune responses of AM. Materials and Methods: We studied the *in vivo* effects of PM on human anti-mycobacterial host immunity in AM obtained from healthy subjects living in Mexico City. We also evaluated whether PM induces *in vitro* changes in AM INFγR, CD14, CD64 and CD206membrane receptors, which are important in the protective response against *Mtb* infection. At the same time we evaluate the induction of the arylhydrocarbon receptor (AhR), that senses environmental toxins like PM. Results: PM reduced CD64 expression and increased CD206 and AhR expression, which suggests that the protective response against Mtb may be altered. The INFγR and CD14 receptors were not affected in response to PM. Conclusions: PM exposure affects immunity response against *Mtb* in AM. AM activation may be altered by decreased expression of CD64 while the anti-inflammatory response is favored by the expression of CD206 and AhR.

P.A5.01.05
Intravital imaging of neutrophil dynamics after LPS administration

N. Chen*, E. v. Grinsven, L. Koenderman, N. Vrisekoop;*
Laboratory of Translational Immunology, Utrecht, Netherlands.

Upon acute inflammation evoked by intravenous infusion of lipopolysaccharide (LPS) in healthy human volunteers, neutrophil numbers in the blood initially decrease the first 90 min and subsequently dramatically increase. During this neutrophilia extra neutrophil subsets appear in the blood that can be distinguished based on nuclear morphology and expression of CD16 and CD62L. Banded CD16^{low} as well as hypersegmented CD62^{low} neutrophils appear in the blood.
In this study we aimed to establish the mechanism behind the neutropenia found during the first 90 minutes after LPS administration. In addition we are investigating whether CD62L^{low} neutrophils i) shed CD62L directly in the blood stream, ii) loose CD62L upon rolling along the blood vessel, or iii) whether CD62L^{low} neutrophils are recruited to the blood from another location.
Intravital microscopy has revolutionized biomedical research during the last two decades and has been used extensively to study dynamic process in intact tissues of living animals. Here we intravitally image neutrophil dynamics and CD62L expression in blood vessels of the mouse ear upon LPS administration. During the first 90 minutes post LPS administration many neutrophils were found to decrease their speed and crawl along the blood vessels which could explain the lower neutrophil numbers collected in blood withdrawals. Some infrequent extravasation events could also be recorded. After 90 minutes more neutrophils started to appear in the blood stream, closely matching the dynamics found in blood withdrawals of both humans and mice post LPS administration. Upcoming experiments will image CD62L expression of neutrophils upon LPS administration.

P.A5.01.06
Propolis effects on human CD4+ T cells activation by LPS-treated dendritic cells

B. J. Conti[1], *K. B. Santiago*[1,2], *E. O. Cardoso*[1], *F. L. Conte*[1], *L. P. Oliveira*[1], *K. I. Tasca*[1], *M. A. Golim*[3], *M. T. Cruz*[4], *J. M. Sforcin*[1];
[1]*Department of Microbiology and Immunology, Biosciences Institute, UNESP, Botucatu, Brazil,* [2]*Integrated Regional Faculties of Avaré, Avaré, Brazil,* [3]*Laboratory of Flow Cytometry, Blood Center, School of Medicine, UNESP, Botucatu, Brazil,* [4]*Center for Neurosciences and Cellular Biology, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.*

Dendritic cells (DCs) represent a heterogeneous population of professional antigen presenting cells and are essential for recognition and presentation of pathogens to T cells. Propolis is a bee product demonstrating several biological properties, including its immunomodulatory effects. Here, we investigated the modulatory action of propolis in lymphocytes (CD4+ T cells) proliferation by LPS-stimulated DCs. DCs were generated from human monocytes and stimulated with LPS simultaneously or not with propolis for 48h. After, DCs were incubated with CD4+ T cells for 96h to analyze cell proliferation. Cell viability was assessed by MTT assay; DCs phenotype was confirmed by CD14$^-$/CD1a$^+$/CD83^{low} expression and proliferation (CFSE) by flow cytometry. Cell viability was not affected after DCs incubation with LPS or propolis. LPS alone increased CD4+ T cells proliferation whereas its combination with propolis maintained LPS activity in T cells. Since LPS is one of the most potent activators of DCs and able to activate a Th17 profile, propolis may favor its action and drive CD4$^+$ T cells to a pro-inflammatory response. Financial support: FAPESP: 2015/03493-3 and 2015/03409-2

P.A5.01.07
The circadian clock protein BMAL1 regulates IL-1β in macrophages via NRF2

J. O. Early[1], *D. Menon*[1], *C. Wyse*[2], *M. Cervantes*[2], *Z. Zaslona*[1], *D. Ryan*[1], *L. A. O'Neill*[1], *A. M. Curtis*[2];
[1]*Trinity biomedical sciences institute, Dublin, Ireland,* [2]*Royal College of Surgeons, Dublin, Ireland.*

The molecular clock, also termed the circadian clock, is the timekeeping system within all our cells that integrates many aspects of our biology to align with the 24 hr external environment. Cells of the innate immune system, including macrophages, have a robust molecular clock. BMAL1 is the major regulator of the molecular clock and its deletion in macrophages leads to a pro-inflammatory phenotype. However the molecular mechanisms by which the molecular clock controls inflammation are not fully known. We have discovered that BMAL1 is crucial in triggering antioxidant defence in macrophages. Using a systems level approach, we demonstrate that macrophages lacking *Bmal1* (and thus lacking a functional molecular clock) have diminished activity of NRF2, a key antioxidant transcription factor. *Bmal1* knockout macrophages have reduced levels of NRF2 protein and lower induction of NRF2 target genes including *Hmox-1* and *Gsr* following lipopolysaccharide (LPS) stimulation. The master antioxidant, glutathione, whose synthesis relies on NRF2 activity, is also severely reduced in cells lacking *Bmal1*.

Such impairments in NRF2 mediated antioxidant defense result in increased production of reactive oxygen species (ROS) in *Bmal1-/-* macrophages. Deletion of *Bmal1* boosts the transcription, translation and cleavage of the pyrogen IL-1β, a cytokine well documented to be under regulation by ROS and NRF2. We have shown that use of antioxidants or enhancement of NRF2 activity can rescue the pro-inflammatory phenotype of *Bmal1-/-* macrophages. These findings uncover a novel mechanism by which the circadian cycle can control disease progression in inflammatory diseases such as sepsis, asthma and rheumatoid arthritis.

P.A5.01.08

Liver injury after acute CCL_4administration is independent of Smad7 expression in myeloid cells

J. Endig[1], *D. Goltz*[2], *P. Sprezyna*[1], *L. Diehl*[1];
[1]*Institute of Experimental Immunology and Hepatology, Hamburg, Germany,* [2]*Institute of Pathology, Bonn, Germany.*

Myeloid cells are essential for liver homeostasis, initiation and determination of innate and adaptive immunity. Smad7 is an inhibitor of the transforming growth factor-β signalling pathway that not only regulates T cell differentiation but also inflammatory cellular processes. Knockdown of Smad7 in hepatocytes has been shown to promote liver fibrosis, but little is known about the effects of Smad7 in myeloid cells during inflammatory responses in the liver. Using mice with a myeloid specific knock-down of Smad7 (LysM-CrexSmad7$^{fl/fl}$) we investigated the impact of Smad7-deficiency in myeloid cells on liver inflammation and regeneration using the well-established model of CCl_4-mediated liver injury. Early (24/48h) and late (7d) time-points were analysed. We found that CCl_4 induces a severe liver injury, with elevated serum ALT levels, centrilobular and periportal necrosis, infiltrating myeloid cells, and an increase of inflammatory cytokines in the liver. Furthermore, as expected, inflammation peaked at 24h and subsided after 7d. However, there were no significant differences in the investigated parameters between the Smad7 wild type and Smad7 knock-down treatment groups. Thus our results suggest that inhibiting TGFβ signalling via Smad7 expression in myeloid cells is dispensable for the induction and control of liver injury.

P.A5.01.09

The role of inflammasomes in human dendritic cell subsets

L. Hatscher[1], *L. Heger*[1], *C. H. Lehmann*[1], *J. J. Lühr*[1], *L. Amon*[1], *G. F. Heidkamp*[1], *A. Hartmann*[2], *I. Ivanović-Burmazović*[3], *A. Purpojo*[4], *R. Cesnjevar*[4], *O. Groß*[5], *D. Dudziak*[1];
[1]*Department of Dermatology, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nürnberg (FAU), Erlangen, Germany,* [2]*Department of Pathology, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nürnberg (FAU), Erlangen, Germany,* [3]*Department of Chemistry and Pharmacy, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany,* [4]*Department of Pediatric Cardiac Surgery, University Hospital Erlangen, Friedrich-Alexander- University Erlangen-Nürnberg (FAU), Erlangen, Germany,* [5]*Institute of Neuropathology, Medical Center - University of Freiburg, Freiburg, Germany.*

Dendritic Cells (DCs) are potent antigen presenting cells and required for connecting innate to adaptive immune responses. Therefore inflammasomes, important for the defense against pathogens or for sensing many different pathogen-associated and danger-associated molecular patterns, play a crucial role in DC mediated immune responses. Growing evidence suggests an important function of inflammasomes in murine DCs but the specific role in the distinct human DC subsets has yet to be determined. In this ongoing study we show that stimulation of human CD1c$^+$ DCs with inflammasome ligands after TLR engagement induces the secretion of TH1 and TH17 polarizing cytokines, potentially resulting in an enhanced T cell response in contrast to TLR stimulation alone. Stimulation of CD1c$^+$ DCs with activators of NLRP1 or NLRP3 after TLR 7/8 engagement showed strong secretion of IL-1β, IL-12 and IL-23. Moreover, applying caspase-1 inhibitors effectively inhibited IL-1β secretion and therefore inflammasome and caspase activation. Activation of inflammasomes is frequently accompanied by pyroptosis, an inflammatory form of programmed cell death. Activating inflammasomes while maintaining cell viability was termed hyperactivation and has been described for OxPAPC, a mixture of oxidized phospholipids, in murine BMDCs. LDH release assays revealed only a slight or no cytotoxic effect for a few inflammasome activators in contrast to classical NLRP3 inflammasome activators such as ATP or Nigericin. Taken together, our results suggest promising effects for particular inflammasome ligands as potential hyperactivating adjuvants for boosting DC mediated immune responses.

P.A5.01.10

Activation of M(IFN-gamma) macrophages by *Porphyromonas gulae* LPS

J. A. Holden, *J. C. Lenzo, N. M. O'Brien-Simpson, E. C. Reynolds;*
Oral Health CRC, The University of Melbourne, Melbourne, Australia.

Porphyromonas gulae is an anaerobic Gram-negative bacterium that has been associated with periodontal disease in companion animals. Lipopolysaccharide (LPS) is a known virulence factor in bacteria such as *Porphyromonas gingivalis*, a closely related bacterium associated with chronic periodontitis in humans. However, the activity of *P. gulae* LPS has yet to be investigated. *P. gulae* LPS induced a similar level of TLR4 activation compared to *P. gingivalis* LPS, both significantly impaired compared to *E. coli* LPS. *P. gulae* and *P. gingivalis* LPS also had TLR2 activity; which could be partially reduced with lipoprotein lipase. *P. gulae* and *P. gingivalis* LPS activated interferon-gamma treated macrophages as evidenced by CD86 expression and low levels of nitric oxide synthesis, which was dependent on TLR2 signalling and partially dependent on the TLR4 signalling pathway. *P. gulae* LPS induced higher levels of inflammatory cytokines than *P. gingivalis* LPS, comparable to that induced by *E. coli* LPS, which was completely sensitive to deletion of the TLR2 signalling ability. These results demonstrate *P. gulae* may have a modified lipid A moiety and thus avoids TLR4 activation, similar to *P. gingivalis*.

P.A5.01.11

Gene expression profiles of human resident lamina propria dendritic cells predict a functional role in tissue repair and angiogenesis under inflammatory conditions

S. Kurzhals[1], *S. Schiessling*[2], *T. Giese*[1], *G. Wabnitz*[1], *A. Heidtmann*[1], *F. Lasitschka*[3], *M. AlSaeedi*[4], *M. Schneider*[4], *S. Meuer*[1], *J. Schröder-Braunstein*[1];
[1]*University Hospital Heidelberg, Institute of Immunology, Heidelberg, Germany,* [2]*Klinikum Oldenburg, Oldenburg, Germany,* [3]*University Hospital Heidelberg, Institute of Pathology, Heidelberg, Germany,* [4]*University Hospital Heidelberg, Heidelberg, Germany.*

Human intestinal lamina propria dendritic cells (LPDC) are known to be important regulators of intestinal adaptive immune responses. By extending dendrites through the intestinal epithelial layer they are capable of sampling intestinal luminal antigens. They subsequently transport these antigens to the mesenteric lymph nodes where they induce the activation and differentiation of antigen specific T-lymphocytes.
In contrast to their impact on intestinal adaptive immune responses, local functions of lamina propria DCs during intestinal inflammation remain mostly unknown. Using a human intestinal organ culture model, we demonstrate that -under inflammatory conditions- CD45$^+$, lineage$^-$, CD14$^-$, CD11c$^+$ myeloid LPDC are not only able to migrate out of the lamina propria onto the luminal side of the basement membrane following epithelial cell depletion. They also express higher levels of genes encoding inflammatory cytokines and chemokines such as *IL6, IL1B,* and *CCL22* as well as of mediators associated with tissue repair (*MMP12, CTGF, BMP6*) and angiogenesis (*VEGFA*) when compared to PB-DC. Interestingly, we observed increased mRNA levels of the IL-12 family cytokine subunits *IL23A* and *EBI3* while the mRNA levels of the corresponding binding partners IL12-p40 (for IL23p19), IL12-p35 and IL27-p28 (for EBI3) were not significantly increased. IL23p19 and EBI3 could potentially form the newly discovered IL-12 family member IL-39. According to recent studies IL-39 is involved in the regulation of inflammation by promoting neutrophil differentiation/expansion as well as wound healing.
These results implicate that human resident LP-DC are not only regulating innate and adaptive immune responses but have a much broader spectrum of tasks.

P.A5.01.12

The role of CR3 (CD11b/CD18) and CR4 (CD11c/CD18) in adherence under physiological and inflammatory conditions

S. Lukácsi[1,2], *T. Gerecsei*[3,4], *B. Francz*[5], *B. Szabó*[3], *R. Horváth*[4], *A. Erdei*[1,2], *Z. Bajtay*[1,2];
[1]*Department of Immunology, Eötvös Loránd University, Budapest, Hungary,* [2]*MTA-ELTE Immunology Research Group, Eötvös Loránd University, Budapest, Hungary,* [3]*Department of Biological Physics, Eötvös University, Budapest, Hungary,* [4]*Nanobiosensorics "Lendület" Group, Institute of Technical Physics and Material Sciences, Centre for Energy Research, Hungarian Academy of Sciences, Budapest, Hungary,* [5]*CellSorter Company for Innovations, Budapest, Hungary.*

CR3 and CR4 belong to the family of β_2-integrins and play an important role in phagocytosis, cellular adherence and migration. CR3 and CR4 are generally expected to mediate similar functions due to their structural homology and overlapping ligand specificity. Previously we proved that CR4 is dominant in the adhesion of monocytes, monocyte derived macrophages (MDMs) and monocyte derived dendritic cells (MDDCs) to fibrinogen under physiological conditions. Here we studied the expression of CR3 and CR4 and their participation in adherence to fibrinogen in inflammatory conditions induced by LPS.
The expression of CR3 and CR4 was monitored by flow cytometry at different time points during the LPS induced cell activation. Cell adhesion to fibrinogen was evaluated using a state-of-the-art biophysical method, namely the force of cell attachment was measured with a computer controlled micropipette using vacuum assisted fluid flow.
Comparing the amount of CR3 and CR4 on the cell surface we found that LPS treatment alters their expression differently on MDMs and MDDCs. Whereas on MDMs the expression of both CR3 and CR4 decreases (44%±4 and 64%±17, respectively compared to non-treated control cells), on MDDCs CR4 shows a significantly elevated level (up to 147% ±34), in contrast to the diminished expression of CR3 (73%±10). Applying the micropipette, we observed a reduced adhesion force in the case of the LPS treated cells, that was more pronounced in MDDCs than in MDMs. Using blocking antibodies we proved that adherence to fibrinogen is dominated by CD11c in both physiological and inflammatory conditions.

P.A5.01.13

Metabolic conversion of IP_3 to IP_4 is required to operate Ca^{2+} entry and NFAT activation in dendritic cells in response to LPS

L. Marongiu[1], *F. Mingozzi*[1], *M. Di Gioia*[2], *L. Sironi*[1], *M. Garrè*[3], *D. Parazzoli*[3], *T. Morii*[4], *C. Cigni*[1], *R. Marzi*[1], *R. Rotem*[1], *M. Colombo*[1], *D. Prosperi*[1], *I. Zanoni*[2,1], *F. Granucci*[1];
[1]*University of Milano Bicocca, Milano, Italy,* [2]*Harvard Medical School and Division of Gastroenterology, Boston Children's Hospital, Boston, MA, United States,* [3]*IFOM, the FIRC institute of Molecular Oncology, Milano, Italy,* [4]*Institute of Advanced Energy, Kyoto University, Kyoto, Japan.*

Introduction. Following LPS exposure different NFAT members are activated in DCs. The initiation of the pathway that leads to nuclear NFAT translocation is dependent on CD14 that, through the activation of Src family kinases and PLCγ2, leads to Ca^{2+} mobilization and calcineurin activation. Nuclear NFAT translocation is required for IL-2 production and apoptotic cell death of terminally differentiated DCs. Here we analyzed the mechanism of Ca^{2+} mobilization in DCs.
Results. We demonstrated that mouse and $CD14^+$ human DCs expressed IP3R3 at the plasma membrane and that these receptors colocalized with CD14 in lipid raft. We found that the increase in cytosolic calcium concentration was due to a direct calcium influx from the extracellular space, with a mechanism dependent on IP_3R3, and required the production of IP4. Indeed, abrogation of IP3R3 or IP3KB in DCs also abrogated calcium mobilization and NFAT activation. Moreover, pharmacological inhibition of IP3KB restrains inflammatory events (increased vessel permeability or inflammatory arthritis) regulated by NFATs in the presence of LPS, similar to the direct inhibition of NFATs by nanodrugs.
Conclusions. Our results indicate that the mechanism of calcium influx triggered by CD14 requires the activation of IP3KB and therefore the metabolic conversion of IP_3 to IP_4. The release of IP4 close to the plasma membrane leads to the opening of IP_3R3 with the consequent induction of a monophasic Ca^{2+} influx necessary to activate the downstream events that trigger NFAT dependent transcriptional program. Therefore, IP3KB represents a new target for anti-inflammatory therapies aimed at inhibiting specific DC functions.

P.A5.01.14

Human neutrophil-derived extracellular vesicles promote Th17 cell development

S. Mol[1,2], *E. W. Taanman-Kueter*[1], *M. H. Wauben*[2], *E. C. de Jong*[1], *T. Groot Kormelink*[1];
[1]*Department of Experimental Immunology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands,* [2]*Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands.*

Th17 cells are important effector cells in fighting extracellular pathogens and are crucial players in the pathology of autoimmune disorders. We have recently demonstrated that neutrophil-derived elastase is essential for DC-driven Th17 cell development. However, it is yet unknown whether the uptake of elastase by DCs is important, and if extracellular vesicles (EVs) are involved in this process. In this study we investigate the role of human neutrophil-derived EVs in the transfer of elastase and the induction of Th17 cells. Neutrophils were isolated from blood of healthy donors and cultured in the absence or presence of activating stimuli: TNFα, LPS, fMLP. After stimulation, cells were collected and survival and activation were analyzed using flow cytometry. EVs were isolated from supernatants by differential centrifugation. For quantification by high-resolution flow cytometry, this step was followed by density ultracentrifugation. We found that neutrophil activation by TNFα and LPS did not enhance EV release compared to non-activated neutrophils. Surprisingly, fMLP activation resulted in the release of less EVs. Next we evaluated the capacity of neutrophil-derived EVs (EV-enriched 100,000xg pellets) to induce Th17 cell development from naïve CD4+ T cells in co-cultures with *C. Albicans*-activated monocyte-derived dendritic cells. Preliminary data suggests that EVs derived from TNFα/LPS-activated neutrophils, but not EVs from non-activated and fMLP-activated neutrophils, enhance Th17 cell development. These results indicate that activated neutrophils release EVs that promote DC-driven Th17 cell development. Our findings highlight a yet undescribed mechanism on the control of adaptive immunity by innate immune cell-derived EVs.

P.A5.01.15

LPS-induced neutrophils activation is modulated by new anti-PDE4 compounds

A. Moniot[1], *C. Guillaume*[1], *I. Allart-Simon*[2], *J. Sapi*[2], *S. Gérard*[2], *S. C. Gangloff*[1], *F. Velard*[1];
[1]*EA4691 Bios, Reims, France,* [2]*UMR CNRS 7312 ICMR, Reims, France.*

Polymorphonuclear Neutrophils (PMNs) are inflammatory cells, whose activation leads to abundant secretion of interleukin-8 (IL-8) and matrix metalloprotease 9 (MMP-9). To limit exacerbated cells response and tissue damages, anti-inflammatory molecules are commonly used. In this field, novel therapeutics are constantly sought to increase the anti-inflammatory arsenal. Type IV phosphodiesterase (PDE-IV) is able to hydrolyze cyclic adenosine monophosphate (cAMP) into AMP. PDE-IV related activity is increased in inflammatory processes. In this context, the PDE-IV inhibitors have proved to be particularly promising.
In the here-presented *in vitro* work, we have tested five pyridazinone scaffold-based PDE-IV inhibitors for their capability to modulate intracellular cAMP and the expression of IL-8, MMP-9 and TNF-α by human neutrophils.
Our results confirmed that pro-inflammatory stimulus (LPS) induced a decrease in intracellular cAMP level, this decrease has been counteracted by all our molecules. None of them impaired either mRNA expression (RT-qPCR) or protein secretion (ELISA, zymography) in basal condition. In pro-inflammatory condition, IL-8, TNF-α and MMP-9 concentration was increased in cell culture supernatant (three-fold, thirty-fold and two-fold respectively, $p<0.05$) whereas no variation has been seen at the mRNA level. Of interest, zardaverine (positive control for PDE-IV activity inhibition) and our PDE-IV inhibitors are able to decrease IL-8 and TNF-α secretion by at least 20% and 65% versus LPS condition respectively, as well as MMP-9 activity by 33%.
Taking together, our data reveal that pyridazinone derivatives may be interesting candidates as therapeutics against inflammatory diseases as they demonstrated anti-PDE-IV activity and seemed to be able to limit neutrophils activation.

P.A5.01.16

HLA-DR, CD4 and CD45-RO in neutrophil extracellular traps

F. M. Rodriguez, ***I. Novak****;*
Institute of Cell Biology, Faculty of Medicine, Cordoba, Argentina.

Introduction: Neutrophil polymorphonuclear leukocytes (PMN) may express costimulatory B7 molecules: CD80 and CD86 and molecules of MHC II: HLA-DR after stimulation with lipopolysaccharide (LPS), this could allow them to acquire competence as a professional antigen presenting cell (APC). Early few works described expression of CD4 and CD45RO in human peripheral blood neutrophils with modulatory functions. Neutrophil extracellular traps (NETs) formed by chromatin and granular proteins are structures released by PMN under inflammatory conditions. In our previous work, the presence of CD80 and CD86 costimulatory molecules in NETs was described. Objectives: generation of NETs in PMN cultures, challenged with LPS and perform marking HLA-DR, CD80 and CD86. In NETs from PMN cultures and from co-cultures of PMN stimulated with LPS and mononuclear (MN) stimulated with OVA perform marking CD4 and CD45-RO. Methods: purified PMN and MN were obtained by density gradient centrifugation and autologous leukocyte cultures from healthy human blood samples with ethical consent, anticoagulated with heparin. PMNs were stimulated with LPS and MNs with OVA. Samples: 30 min. Immunofluorescence technique with anti-CD80, anti-CD86, anti HLA-DR, anti CD4, anti CD45-RO Abs, DNA stain with DAPI. Paired blood samples provided controls. Results: CD80, CD86 and HLA-DR molecules were observed in NETs. In co-cultures experiments with autologous MNs stimulated with OVA, positivity was also observed for CD4 and CD45-RO in NETs. Conclusions: the expression of molecules normally associated with APCs and, on the other hand, CD4 and CD45RO expression in NETs allow to think more roles of PMN in innate and adaptive immunity.

P.A5.01.17

Differential imprinting of $CD4^+$ T cell lung-homing capacity by conventional dendritic cells from inguinal and mediastinal lymph nodes

D. PEJOSKI[1,2,3], *P. Fontannaz*[1,2,3], *S. Grillet*[1,2,3], *D. Christensen*[4], *P. Andersen*[4], *P. Lambert*[1,2,3], *C. Siegrist*[1,2,3];
[1]*Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland,* [2]*World Health Organization Collaborating Center for Vaccine Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland,* [3]*Center for Vaccinology, Geneva University Hospitals, Geneva, Switzerland,* [4]*Statens Serum Institut, Copenhagen, Denmark.*

Novel adjuvants that instruct protective T cells to home to desired anatomical sites could be used to improve vaccine efficacy, for example against respiratory pathogens such as *Mycobacterium tuberculosis*. Dendritic cells (DCs) that drain different anatomical sites are capable of delivering tissue-specific 'imprinting' signals during T cell priming, which favor the establishment of T cell populations that home to the original site of DC-antigen (Ag) encounter. The imprinting signals provided by DCs have been defined for several tissues though it is unclear whether a similar imprinting phenomenon occurs for lung trafficking T cells. We therefore used a Poly(I:C) adjuvant and protein Ag vaccine model to compare airway and muscle-draining DCs in terms of phenotype and their ability to imprint T cell lung-homing markers. Our results demonstrate that Ag^+ $CD11b^+$ and $CD103^+$ conventional DC subsets are found in both intramuscular (i.m.)-draining inguinal lymph nodes (LNs) and intranasal (i.n.)-draining mediastinal LNs (ILN, MLN) after immunization. Importantly, cell-sorted Ag^+ $CD11b^+$ DCs from the MLN were superior, on a per-cell basis, to ILN-derived DCs at *in vitro* priming of $CD4^+$ T cells with a lung-homing phenotype. The MLN-DC primed T cells then showed an enhanced capacity for *in vivo* trafficking to the lung parenchyma when transferred into naïve mice, demonstrating that site-specific DC imprinting of lung-homing T cells occurs in this immunization model. Grant declaration: This work was supported by a Horizon2020 fund for TBVAC2020 project PHC-08-2014, paid by the Swiss Confederation SEFRI Contract 15.0033-4, 643381

P.A5.01.18

Glycan-binding profiles of human and murine dendritic cells

***E. Rapoport**, E. Moiseeva, S. Khaidukov, D. Aronov, G. Pazynina, S. Tsygankova, N. Bovin;*
Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russian Federation.

Modification of vaccine particles with glycan promotes binding to dendritic cells (DCs) resulted in augmenting of vaccine efficacy. Therefore, we used a library of 229 fluorescent glycoprobes (Glyc-PAA-fluo) to select potential glycan "vector". We found that the highest percent of probe-positive $CD14^{low/-}CD16^{+}CD83^{+}$ subpopulation containing blood circulating DCs was observed for GalNAcα1-2Galβ (A_{di}), $(Neu5Acα2-8)_3$ and for three mannose-reach glycans, namely (Galβ1-4GlcNAcβ1-2Manα)$_2$-3,6-Manβ1-4GlcNAcβ1-4GlcNAcβ, Manα1-3(Manα1-6)Manβ and Manα1-3(Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ. Mouse models are widely used to characterize different DCs subpopulations *in vivo* and *in vitro* as comparative genomics revealed functional equivalences between distinct human and mouse DCs subsets. The aim of this work was to compare glycan-binding profiles of circulating in human and murine blood DCs. Murine circulating DCs were identified as $CD11c^{+}CD14^{low/-}CD16^{+}CD80^{+}$ in the blood of BYRB male mice. High percent of positive bound murine $CD14^{low/-}CD16^{+}CD80^{+}$- cells were observed for A_{di}, $(Neu5Acα2-8)_3$ and Manα1-3(Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ. The other probes selected for human $CD14^{low/-}CD16^{+}CD83^{+}$ cells did not display any binding to murine $CD11c^{+}CD14^{low/-}CD16^{+}CD80^{+}$ cells. Thus, glycan-binding profiles of murine and human DCs are not identical that should be taken into account when the glycovaccines are tested on mice *in vivo*. This work was supported by grant 16-04-01084 of the Russian Foundation for Basic Research and EU FP7 Project UniVax (Health -F3-2013-60173), grant number 601738.

P.A5.01.19

DOCK8 regulates macrophage migration through Cdc42 activation and LRAP35a interaction

***T. Sakurai**[1], A. Shiraishi[1], Y. Fukui[1,2];*
[1]Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan, [2]Reseach Center for Advanced Immunology, Kyushu University, Fukuoka, Japan.

Introduction: DOCK8 is an atypical guanine nucleotide exchange factor for Cdc42, and its mutations cause combined immunodeficiency in humans. Accumulating evidence indicates that DOCK8 regulates the migration and activation of various subsets of leukocytes, but its regulatory mechanism is not completely understood. Material and methods: Bone mallow-derived macrophages from $DOCK8^{+/-}$ or $DOCK8^{-/-}$ mouse were used for chemotaxis assay, immunofuruorescence staining, and biochemical analyses. Protein binding was assessed by immunoprecipitation, pull-down assays, and immunoblotting. Results: DOCK8-deficient macrophages exhibited a migration defect in a 2D setting. Although DOCK8 deficiency did not affect the global Cdc42 activation in macrophages, rescue experiments revealed that the guanine nucleotide exchange factor activity of DOCK8 was required for macrophage migration. We found that DOCK8 associated with LRAP35a, an adaptor molecule that binds to the Cdc42 effector myotonic dystrophy kinase-related Cdc42-binding kinase (MRCK), and facilitated its activity to phosphorylate myosin II regulatory light chain (MLC2). When this interaction was disrupted in wild-type macrophages, they showed a migration defect, as seen in DOCK8-deficient macrophages. Conclusion: DOCK8 links Cdc42 activation to actomyosin dynamics through the association with LRAP35a during macrophage migration.

P.A5.01.20

Effects of propolis on human CD4+ T cells proliferation induced by MAGE-1-treated dendritic cells

***K. B. Santiago**[1,2], B. J. Conti[1], E. O. Cardoso[1], L. P. Oliveira[1], F. L. Conte[1], K. I. Tasca[1], M. A. Golim[3], M. T. Cruz[4], J. M. Sforcin[1];*
[1]Department of Microbiology and Immunology, Biosciences Institute, UNESP, Botucatu, Brazil, [2]Integrated Regional Faculties of Avaré, Avaré, Brazil, [3]Laboratory of Flow Cytometry, Blood Center, School of Medicine, UNESP, Botucatu, Brazil, [4]Center for Neurosciences and Cellular Biology, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.

Dendritic cells (DCs) are professional phagocytic cells that play an important role in the immune response and T lymphocytes stimulation. Propolis is a bee product that has been used since ancient times due to its many biological properties, such as the immunomodulatory action. This work aimed to investigate the modulatory action of propolis in T cells proliferation stimulated by DCs incubated with a tumor antigen (human MAGE-1) identified in melanoma and other types of tumors. Human monocytes and lymphocytes were obtained from healthy donors (n = 5) and isolated with magnetic beads. DCs were generated from human monocytes and stimulated with human MAGE-1 (10 µg/mL) simultaneously or not with propolis (5 µg/mL) for 48 h. After, DCs were incubated with T cells for 96 h to evaluate their proliferation. Cell viability was analyzed by MTT assay; DCs phenotyping ($CD14^{low}/CD1a^{high}/CD11c^{high}/CD83^{low}$) and T cell proliferation (CFSE) by flow cytometry. Significant differences were determined by analysis of variance followed by the Dunnett's test ($P < 0.05$). Propolis in combination or not with MAGE-1 did not affect DCs viability. Propolis alone increased T cell proliferation, and its combination with MAGE-1 presented a higher proliferation rate compared to control. Propolis exerted an immunomodulatory action in the presence of MAGE-1, suggesting that it leads to $CD4^{+}$ T cells activation and may favor a Th1 profile and consequently activation of cellular immunity. Financial support: FAPESP (2015/02596-3 and 2015/03493-3).

P.A5.01.21

Endocytosis of particulate matter of neutrophils induced oxidative stress through dynamin

***y. yoshida**, T. Miyake, D. Wang, M. Shen, K. Morita;*
Univ.Occup.& Environ. Health, Dep. Imm., Kitakyushu, Japan.

We previously reported the biological effects of PM in vivo, however, few reports have focused on the relationship between PM inhalation and neutrophils. Here, we investigated the effect of PM particle size on neutrophils. Flow cytometry analysis indicated that 1 µm particles are readily endocytosed by neutrophils and that endocytosis is reduced at 4°C. Inhibitors of the pleckstrin homology domain of dynamin repressed this process; however, GTPase and clathrin inhibitors did not affect endocytosis. Endocytosis by neutrophils in Toll-like receptor 4 (TLR4)- and MyD88-knockout mice was reduced compared with that in wild-type mice, indicating that TLR4 and MyD88 are important for the process. Neutrophil-mediated endocytosis caused oxidative stress, and N-acetylcysteine enhanced endocytosis. Expression levels of the oxidative stress markers, heme oxygenase-1 and p62 protein, were increased in an endocytosis-dependent manner. Phagocytosed neutrophils produced IL-6 and TNFα, whose production was decreased by dynamin inhibitors. Overall, these results indicate that endocytosis and ROS production via TLR4 are important for the initiation of immune responses by neutrophils.

P.A5.02 Initiation of immune responses - Part 2

P.A5.02.01

Resolution of inflammation is altered in multiple sclerosis

***V. Chiurchiù**[1,2], A. Leuti[1,2], P. Norris[3], I. Riley[3], M. Albanese[4], L. Battistini[5], C. Serhan[3];*
[1]Neurochemistry of Lipids, European Center for Brain Research, Santa Lucia Foundation, Rome, Italy, [2]Department of Medicine, Campus Bio-Medico University of Rome, Rome, Italy, [3]Brigham and Women's Hospital, Harvard Medical School, Boston, United States, [4]Neurology Unit, Department of Neurosciences, University of Rome Tor Vergata, Rome, Italy, [5]Neuroimmunology Unit, European Center for Brain Research, Santa Lucia Foundation, Rome, Italy.

Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disease associated to uncontrolled inflammation and autoimmunity. Recent studies suggest that these can be a consequence of failure to resolve inflammation, a process that strictly depends on a newly discovered genus of highly potent anti-inflammatory lipids derived metabolically from omega-3 essential fatty acids and termed specialized pro-resolving lipid mediators (SPMs), that include resolvins, lipoxins and protectins. Here, by means of targeted metabololipidomics, we found that levels of specific SPMs such as lipoxins A4 and B4 as well as resolvin D1 (RvD1) and protectins PD1 and PDX were increased in MS patients during the relapse phase, with RvD1 and PD1 being also reduced or undetected in the progressive phase. Principal Component Analysis (PCA) revealed differences in the production of specific pro-inflammatory mediators and SPMs according to the disease phase. Variations in the plasma levels of SPMs also significantly correlated with clinical scores and were also paralleled by contradistinctive expression of SPM biosynthetic enzymes (COX-2, LOX-5, LOX-15) and receptors (ALX/FPR2, GPR32, GPR18) in blood leukocytes. When treating peripheral blood leukocytes of relapse MS patients with several SPMs, we observed a significant effect in reducing their inflammatory profile in terms of cell activation and cytokines production. These findings show for the first time a dysfunction of the resolution pathway in the blood of MS patients, accounting for a possible role of SPMs as leads for the control of MS pathogenesis and progression by modulating key effector cells in the periphery.

P.A5.02.02

The T-cell response in ferrets against influenza A virus is influenced by the site of infection

*K. van de Ven, H. van Dijken, F. de Heij, **J. de Jonge**;*
National Institute of Public Health and the Enviro, bitlhoven, Netherlands.

Influenza A virus (IAV) infects millions of people each year, resulting in respiratory disease with symptoms ranging from a mild common cold to a severe fatal viral pneumonia. Vaccines may protect against multiple IAV subtypes by targeting conserved intracellular epitopes of IAV. By using ferrets, we can assess how the T-cell response against IAV is influenced by the site of induction, which IAV proteins are more likely to evoke an immune response and which proteins are involved in cross-protection. In a recent study, we infected ferrets (n=28) intranasal (i.n.) or intratracheal (i.t.) with H2N2 or PBS and analyzed samples from pre-infection and 14 days post infection. We found that i.n. infection with H2N2 invoked a stronger virus-specific T-cell response in the blood.
However, more CD8+ T-cells could be detected in the bronchoalveolar lavage of i.t. infected ferrets. T-cells showed strong responses against peptides of the conserved H2N2 proteins PA, PB1 and PB2, which corresponds with our observation that T-cells of H2N2 infected animals cross-react to H1N1. These results imply that the site of vaccination influences the T-cell response, which can contribute to the development of more efficient IAV vaccines against seasonal and pandemic IAV.

P.A5.02.03

Complement deficiency attenuated multiple organ failure in zymosan induced aseptic shock

P. Ganova[1], *V. Gyurkovska*[1], *L. Belenska-Todorova*[2], *N. Ivanovska*[1];
[1]*Institute of Microbiology, Sofia, Bulgaria,* [2]*Medical Faculty, Sofia University, Sofia, Bulgaria.*

Septic shock is a complex inflammatory disease associated with a high rate of mortality. It starts with an overwhelmed immune response to infectious agents or their products in which the activated macrophages, neutrophils and the complement system play important roles. Cytokines and inflammatory mediators produced and secreted at first hours can induce organ failure and damage. Shock was induced by intraperitoneal injection of 1 mg/g body weight of zymosan in BALB/c mice. Functional complement activity was exhausted by cobra venom factor (CVF) injection. The peritoneal cells were analyzed by flowcytometry for expression of monocyte/neutrophil markers and dendritic cells. Plasma samples were analyzed for glucose, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and bilirubin. Livers were conserved in paraffin blocks for histopathological examination. We have observed that complement depletion inhibited zymosan-induced organ dysfunction via decrease of liver injury and changes of hepatic enzyme levels. These effects were also attended with inhibition of spleen and liver enlargement, and by reduced number of pro-inflammatory cells in the peritoneal cavity. Lack of functional complement accelerated GM-CSF-induced bone marrow dendritic cell maturation. The findings presented here show that the development of organ failure can be positively influenced by an inhibition of the functional complement activity. Acknowledgements: This work was supported by a Grant DM 03/4/17.12.2016 National Fund for Scientific Research, Bulgaria

P.A5.02.04

Simulating thrombocyte transfusions to investigate the impact of the self HLA background in HLA antibody formation and the risk for platelet refractoriness

K. Geneugelijk*, T. de Hoop, E. Borst, E. Spierings;*
Laboratory of Translational Immunology, UMC Utrecht, Utrecht, Netherlands.

Platelet refractoriness is a severe condition in which thrombocytic patients fail to achieve sufficient platelet counts after thrombocyte transfusion. This condition can partially be ascribed to alloimmunization towards HLA-A and HLA-B expressed on platelets. However, only a portion of patients will develop HLA antibodies after multiple transfusions. Previous studies have shown that mismatched HLA-derived T-helper epitopes presented by HLA class II (PIRCHE-II) play a role in HLA-antibody formation. The aim of the current study is to investigate whether the patients' HLA background may impact the ability to develop HLA antibodies upon thrombocyte transfusion. To this end, a representative patient population was generated by extracting all HLA-A, -B, -C, -DRB1, and -DQB1 HLA typings that were performed in our center between January 2009 and July 2016. A virtual Caucasian thrombocyte donor population of 10 million individuals was modeled using HLA haplotype frequency tables. This virtual donor population was used to simulate 20 thrombocyte transfusions. PIRCHE-II numbers were calculated for each simulated thrombocyte transfusion. The maximal number of unique T-helper epitopes that patients encountered after 20 simulated thrombocyte transfusions ranged between 31 and 359 epitopes. All patients had encountered half of their maximal T-helper epitopes after only two thrombocyte transfusions. Our simulations show that the maximal number of unique T-helper epitopes after multiple thrombocyte transfusions is highly variable between patients, suggesting a potential role of the self HLA background in the ability to become platelet refractory.

P.A5.02.05

Anthralin-induced skin inflammation is promoted by mast cells

A. Hartmann[1], *J. Sohl*[2], *J. Ringen*[1], *V. Tsvilovskyy*[3], *M. Bros*[4], *H. Schild*[1], *M. Freichel*[5], *T. Feyerabend*[6], *H. Rodewald*[6], *M. Radsak*[2], *M. Stassen*[1];
[1]*Institute for Immunology, University Medical Center, Mainz, Germany,* [2]*3rd Medical Department of the University Medical Center, Mainz, Germany,* [3]*Institute of Pharmacology, University of Heidelberg, Mainz, Germany,* [4]*Department of Dermatology, University Medical Center, Mainz, Germany,* [5]*Institute of Pharmacology, University of Heidelberg, Heidelberg, Germany,* [6]*German Cancer Research Center, Heidelberg, Germany.*

Psoriasis is an inflammatory skin disease characterized by abnormal proliferation of keratinocytes triggered by the cytokines IL-17A and IL-22. Due to its anti-psoriatic and anti-inflammatory action, the natural anthraquinone derivate anthralin is used for the effective treatment of psoriatic plaques without causing severe side effects. Whereas the underlying mechanisms are still not known in detail, anthralin affects growth and proliferation of skin cells. Interestingly, topical treatment with anthralin first enhances skin inflammation before psoriatic plaques begin to heal. In this context, increased mast cells numbers have been shown to be present in psoriatic plaques. Moreover, evidence is accumulating that mast cells contribute to the pathogenesis of psoriasis by secreting IL-17A and IL-22. Focusing the role of mast cells in anthralin-treated skin, we investigated whether mast cells are activated by anthralin *in vitro* and *in vivo. In vitro*, anthralin increases the concentration of intracellular Ca^{2+} in mast cells. Consequently, degranulation of mast cells and production of IL-6 are enhanced in the presence of anthralin. *In vivo*, anthralin causes severe skin inflammation, characterized by enhanced ear swelling, which is delayed in mast cell-deficient Cpa3-Cre mice. Histological staining of murine ear skin showed an enhanced proliferation of epithelial cells and massive mast cell degranulation rates following topical application of anthralin. Taken together, these findings suggest that mast cells contribute to the inflammatory action upon topical anthralin treatment.

P.A5.02.06

Influenza A virus infection during pregnancy: elevated levels of pregnancy hormones alter immune reaction against influenza A virus

A. M. Hierweger[1,2], *G. Engels*[2,3], *K. Thiele*[2], *G. Gabriel*[4], *H. Mittrücker*[1], *P. C. Arck*[2];
[1]*Institute for Immunology, Hamburg, Germany,* [2]*Department of Obstetrics and Fetal Medicine, Laboratory for Experimental Feto-Maternal Medicine, Hamburg, Germany,* [3]*Viral Zoonoses and Adaptation, Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany,* [4]*Viral Zoonoses and Adaptation, Heinrich Pette Institute, Leibnitz Institute for Experimental Virology, Hamburg, Germany.*

During the 2009 H1N1 influenza A virus (IAV) pandemic, pregnant women showed higher influenza related morbidity and mortality. Thus, it is of great clinical interest to investigate the pathogenesis and underlying altered immune responses leading to increased risk for pregnant women.
We previously developed an IAV mouse model where semiallogenically, Balb/c-mated C57BL/6 dams are infected with a 2009 pandemic H1N1 IAV strain. Pregnant mice demonstrate increased mortality and morbidity, which corresponds to the clinical observations in humans.
Using this mouse model, we could show reduced dendritic cell (DC) activation and subsequent reduced CD8 T cell responses in pregnant IAV infected mice compared to non-pregnant littermates. Elevated progesterone levels during pregnancy lead to immune adaptation and could probably account for some of our observations in pregnant IAV infected mice. Therefore, we infected pregnant mice lacking the progesterone receptor in DCs and observed increased survival compared to pregnant infected mice of the control strain. As progesterone can signal via the progesterone and glucocorticoid receptor, mice lacking the glucocorticoid receptor on DCs and T cells will be analysed upon infection. Using an in vitro assay, we could already observe increased apoptosis induction in T cells by progesterone.
These observations strongly support the concept that hormonal changes during pregnancy are relevantly involved in modulation of the immune response against the influenza virus. Whilst the immune modulation during pregnancy is advantageous for pregnancy maintenance it is associated with significant disadvantages for maternal health, mirrored by the increased risk for severe IAV infection.

P.A5.02.07

Cigarette smoke differentially affects inflammatory response depending on exposure time points in a mouse model of nonalcoholic steatohepatitis

J. Kim[1], *Z. Zhou*[1], *H. Jeong*[1], *S. Choi*[2], *S. Lee*[3], *W. Kim*[1], *K. Lee*[2], ***B. Kim***[1];
[1]*Biosafety Research Institute and College of Veterinary Medicine, Chonbuk National University, Iksan, Korea, Republic of,* [2]*Inhalation Toxicology Center, Jeonbuk Department of Inhalation Research, Korea Institute of Toxicology, Jeongeup, Korea, Republic of,* [3]*Division of Biotechnology, College of Environmental and Biosource Science, Chonbuk National University, Iksan, Korea, Republic of.*

Introduction: We tried to demonstrate the impact on main stream cigarette smoke (MSCS) to nonalcoholic steatohepatitis (NASH) progression in sexually matured mice.
Materials and Methods: Mice were either fed a control diet or a methionine-choline deficient with high fat diet for 6 weeks. During the first (early exposure) or last (late exposure) three weeks of diet feeding, each diet group was exposed to MSCS (300 or 600 μg/L: CS300 or CS600) for 2 hours per day and 5 days per week. CS extract (CSE) was extracted from 3R4F reference cigarettes and used for *ex vivo* study.
Results: Hepatic or serum biochemical analysis showed that MSCS differentially modulated hepatic injury in NASH milieu depending on exposure time points. Consistently, histopathologic observation provided that NASH severity was increased in early exposure group, but decreased in late exposure group except for steatosis. Similar results were observed in NASH-related hepatocellular apoptosis and fibrosis as confirmed by TUNEL assay and Sirius red staining, respectively. Our *ex vivo* experiments showed that CSE treatment differentially regulated inflammatory responses in co-cultured hepatocytes and macrophages isolated from liver with steatohepatitis after 10 days or 3 weeks of diet feeding. Furthermore, CSE treatment differentially up- or down-regulated the expression levels of peroxisome proliferator-activated receptor-gamma (PPARγ) in co-cultured macrophages. Finally, CSE treatment differentially affects M1/M2 polarization in co-cultured macrophages.
Conclusions: Our findings indicate that opposite effects of MSCS on NASH progression are mediated by differential modulation of PPARγ and its-associated M1/M2 polarization in hepatic macrophages depending on exposure time points.

P.A5.02.08

Exhaustion and senescence markers in humanized mice confirm the potential use of such model for immunotherapy validation during HIV infection

L. Labarthe[1,2], *S. Henriquez*[1], *O. Lambotte*[1,3], *R. Le Grand*[1], *N. Legrand*[2], *C. Bourgeois*[1];
[1]*CEA - Université Paris Sud 11 - INSERM U1184, Immunology of Viral Infections and Autoimmune Diseases, IDMIT Department, IBFJ, Fontenay-aux-Roses, France,* [2]*Axenis S.A.S, Fontenay-aux-Roses, France,* [3]*Assistance Publique-Hôpitaux de Paris, Service de Médecine Interne et Immunologie Clinique, Groupe Hospitalier Universitaire Paris Sud, Hôpital Bicêtre, Le Kremlin-Bicetre, France.*

Humanized mice harbouring cellular and molecular components of the human immune system (HIS) represent a ground-breaking preclinical platform to study human immune cell behaviour. Before evaluating novel immunotherapies in the context of chronic HIV infection, we aimed to evaluate the exhaustion and senescence profile of human T cells in blood and lymphoid tissues of HIS mice.
In Balb/c $Rag2^{tm1Fwa}$ $Il2rg^{tm1Cgn}$ $Sirpa^{NOD}$ $Flk2^{tm1Irl}$ $HLA\text{-}A2^{Tg\text{-}HHD}$ (BRGSF-A2) HIS mice reconstituted with human cord blood $CD34^{+}$ cells, human hematopoietic cells represented 83% +/- 3,3% in spleen and 68% +/- 15,4% in bone marrow. The human T cell proportion as well as the hCD4: hCD8 T cell ratio were similar to those observed in humans. Naive T cells represented the major T cell compartment in blood and spleen whereas memory T cells were predominant in the bone marrow, as observed in humans. We next studied the basal expression level of exhaustion markers (such as PD-1 and TIGIT, that are important immune checkpoints) and senescence markers (CD57 and KLRG1). Human T cells developing in BRGSF-A2 HIS mice presented an exhaustion level similar to humans, with PD-1 expression being nearly constitutive in the bone marrow, whereas senescence level (as assessed by CD57) was slightly lower.
Overall, the proportion, differentiation, exhaustion and senescence profiles of human T cells in BRGSF-A2 HIS mice resemble those observed in human studies. The physiological expression levels of immune checkpoints in humanized mouse model will allow us exploring anti-immune checkpoints strategies in HIV-1 chronic infection, in order to optimize the current antiretroviral treatment.

P.A5.02.09

Characterization of a conditional and reporter mouse model for the Atypical Chemokine Receptor 2

V. Mollica Poeta[1,2], *M. Massara*[1,3], *R. Carriero*[1], *E. Setten*[1,3], *M. Locati*[1,3], *R. Bonecchi*[1,2];
[1]*Humanitas Clinical and Research Center, Rozzano, Italy,* [2]*Department of Biomedical Sciences, Humanitas University, Pieve Emanuele, Italy,* [3]*Department of Medical Biotechnologies and Translational Medicine, Università degli Studi di Milano, Italy.*

Introduction: Atypical chemokine receptor 2 (ACKR2) is a scavenger receptor able to bind and lead to degradation most CC inflammatory chemokines. Several studies reported its expression on human lymphatic endothelial cells and its implication in chemokine crosstalk that regulate different aspects of lymphatic vessels morphology and activity. Unfortunately, there are no techniques to study ACKR2 expression in mice, due to the lack of a working antibody. The aims of this project are to clarify ACKR2 expression in homeostatic or pathological conditions and to disclose the mechanism by which it could impact on vessels biology. **Methods:** We generated a conditional mouse model with a knock-out/knock-in strategy, by replacing the Ackr2 gene with a TdTomato/Luciferase reporter cassette. By ex vivo imaging system and FACS analysis, we evaluated the expression of ACKR2 in different mouse tissues, especially in the lung. Finally, we performed various lung inflammatory disease models in vivo. **Results:** We found a significant expression of ACKR2 in a restricted pulmonary endothelial cell population previously uncharacterized, that we deeper investigated through RNA-seq analysis. Then, we showed that Ackr2 deficiency in the lung endothelium alter the expression of endothelial cell regulators. We also provided evidence that the lack of ACKR2 had an impact on innate immune response. **Conclusion:** Taking advance of the reporter mouse model development, we clarify the role of the receptor in physiological and pathological context. This will arise the possibility in the future to validate ACKR2 as new target for innovative therapies for lung pathologies.

P.A5.02.10

Association of IL-17A and IL-17F gene polymorphisms with recurrent pregnancy loss in Iranian women

S. Najafi[1], *H. Hadinedoushan*[2], *G. Eslami*[2];
[1]*Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of,* [2]*Yazd University of Medical Sciences, Yazd, Iran, Islamic Republic of.*

Purpose: Recurrent pregnancy loss (RPL) is defined as the occurrence of two or more miscarriages before the 20th week of pregnancy. T helper17 cells are a novel subset of T cells, which secrete IL (Interleukin)-17 and are known to be involved in inflammation, autoimmunity and rejection of non-self tissues. Herein, we studied the association between IL-17A rs2275913 and IL-17F rs763780 gene polymorphisms with RPL in Iranian women.
Methods: A case-controlled study was performed on two groups consisting of 85 healthy women with at least one delivery and 85 women with the history of two or more RPLs. The frequency of IL-17A rs2275913 and IL-17 F rs763780 polymorphisms were determined by PCR-RFLP.
Results: In the RPL group, the genotypes frequencies of rs2275913 polymorphism were GG (8.2 %), AG (30.6 %), and AA (61.2 %) and in the control group, were GG (3.5 %), AG (42.4 %) and AA (54.1 %). Statistical analysis showed no significant difference between the genotypes of AA, AG and GG in the two groups (p=0.1). The genotypes frequencies of rs763780 polymorphism were TT (43.5 %), TC (49.4 %) and CC (7.1 %) in the RPL group; whereas the frequencies were TT (25.9 %), TC (70.6 %) and CC (3.5 %) in the control group. Statistical analysis revealed a significant difference in the TT, TC, and CC genotypes frequencies between the case and the control groups (p=0.01).
Conclusions: Our findings indicate that IL-17F polymorphism, rs763780, might be associated with a high risk of RPL in Iranian women.

P.A5.02.11

Innate and adaptive immune responses in GFP tagged endogenous retroviruses transgenic mice

E. Rauch[1], *M. Hohmann*[1], *T. Amendt*[1], *T. Winkler*[2], *A. Ruhl*[1], *S. Dehnert*[1], *S. Paul*[1], *S. Bauer*[1], *P. Yu*[1];
[1]*Institute of Immunology, Philipps-University Marburg, Marburg, Germany,* [2]*Nikolaus-Fiebiger-Center of Molecular Medicine, Friedrich-Albert University Erlangen- Nürnberg, Erlangen- Nürnberg, Germany.*

The human genome consists largely of inactivated endogenous retroviruses, but their expression and function remain very controversial. In contrast, some mouse strains do express murine ERVs, which are involved in diseases. Recently, it was demonstrated that ERVs of the MuLV type are reactivated in nucleic-acid-sensing TLR deficient and B cell deficient mice. One theory suggests that the increased ERV expression is due to an increased exposure to microbial ligands from the gut. In contrast, our lab suggested an active immune surveillance mechanism suppressing ERV reactivation *in vivo*. TLR7 and in a complementary function TLR3 and TLR9 induce an ERV-specific antibody response.
To study the molecular mechanisms of reactivation of ERVs we generated a novel transgenic mouse, with an endogenous GFP tagged MuLV (EGT-315). Further, we generated EGT-315 mice with TLR379-deficiency to study the viremic pathology. The measurement of the GFP signal from the ERV, indicated reactivation of retrovirus as early as 3 weeks. We observed that the viremia of MuLV-GFP increased in EGT-315 $Tlr7^{-/-}$ mice, whereas in mice with TLR7 the initially lower viremia is suppressed over time. Additionally, TLR7 competent EGT-315 mice produced antibodies against GFP and viral proteins in their plasma, in contrast to TLR7-deficient mice. To investigate the influence of the microbial environment on the activation of MuLV-GFP we stimulated EGT-315 splenic cells with LPS *in vitro*. This lead to a strong increase of MuLV-GFP expression.
Our novel *in vivo* model could be instrumental to understand mechanisms controlling endogenous and exogenous retroviruses.

P.A5.02.12

Prophylactic treatment with a recombinant banana lectin reduce severity of TNBS-induced colitis in BALB/c mice

M. Stojanović[1], *R. Miljković*[1], *A. Filipovic*[1], *I. Lukic*[1], *A. Inić-Kanada*[2], *M. Gavrovic-Jankulovic*[3], *E. Marinkovic*[1];
[1]*Institute of Virology, Vaccines and Sera – Torlak, Belgrade, Serbia,* [2]*Institute of Specific Prophylaxis and Tropical Medicine, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria,* [3]*Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Belgrade, Serbia.*

The recombinant banana lectin (rBL), a structural and functional homolog of natural BL, modulates the immune milieu in the colon of BALB/c mice in a both dose- and time-dependent manner. The enhancement of a production of IL-2 dominates in the initial phase (24h), while the transitional enhancement of a (pro)inflammatory and regulatory cytokines' production occurs later (48h). We aimed to investigate on how the severity of the experimental colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) in rBL-pretreated BALB/c mice correlates with the characteristics of the local immune milieu at the moment of a disease induction. The colitis was induced 24h or 48h upon a treatment with rBL (rectal administration, single dose of 0.5-50 rBL/kg BW). The characterization of the immune milieu at the moment of disease induction was done by determining the local concentration of cytokines (IL-2, IL-12, IL-23, IFNγ, IL-17, IL-6, IL-10 and TGFβ) and abundance of Foxp3+ Treg; severity of the colitis was assessed by measuring the weight loss. We have shown that (i) the reduction in severity of colitis induced 24h upon rBL treatment positively correlates to the local concentration of IL-2 and the abundance of Foxp3+ T cells, while (ii) for colitis induced 48h upon rBL treatment reduction in disease severity positively correlated to the ratio [IL-10]/[IFNγ]. This study also indicated that the reduction in the severity of colitis is not dependent only on rBL dosage but also on the timing of its administration. (Ministry of Education, Science and Technological Development, Republic of Serbia, grant 172049)

P.A5.02.13

Bardet-Biedl Syndrom protein complex in the signaling of the adaptive immune system

***O. Tsyklauri**, V. Niederlová, M. Huranová, O. Štěpánek;*
Institute of Molecular Genetics of the ASCR, v. v. i., Prague, Czech Republic.

BBSome is a transport protein complex, which is important for normal formation and functioning of primary cilium. Mutations in BBSome subunits cause a severe multiorgan disease called Bardet-Biedl Syndrome (BBS). Interestingly, that some of the proteins, which enable ciliary transport, also play a role in the formation of immunological synapse (IS), a contact site between an antigen presenting-cell and a lymphocyte. However, possible impact of BBSome in this process have not been investigated yet. Using YFP-labeled BBS4 subunit, we indicate that BBS4 actually localize to the IS during its formation. In order to investigate possible influence of BBS proteins on the immunity, we established mouse model of BBS (BBS4KO). Comparison of WT and BBS4KO mice confirm expression of BBS4 in normal T- and B-cells. BBS4KO mice have alterations in hematopoietic system, such as increased monocyte number, increased hemoglobin and elevated platelets number. Moreover, our preliminary data indicate that BBS4KO mice have a partial impairment in B-cells development. Further research will shed light on the functions of BBS proteins in immune cells.

P.A5.02.14

IL-33 receptor (ST2) expression on B cells during pregnancy and preterm birth

***N. J. Valeff**[1], L. V. Juriol[1], M. S. Ventimiglia[1], M. C. Abba[2], M. F. Quiroga[3], F. Jensen[1];*
[1]CEFYBO-CONICET, Buenos Aires, Argentina, [2]CINIBA-UNLP, Buenos Aires, Argentina, [3]INBIRS-UBA-CONICET, Buenos Aires, Argentina.

IL-33 is an alarmin released during cell injury caused by stress or infection and drives immune cells into regulatory functions to maintain tissue homeostasis. In the context of pregnancy, IL-33 was recently shown to induce the production of anti-inflammatory molecules by decidual B cells, protecting against preterm labor in human and mouse. Here we aimed to characterize the expression of IL-33 receptor (ST2) in B cells during pregnancy and in preterm birth. We began performing a genome-wide transcriptome profiling in isolated B cells from spleen of pregnant (P) and non-pregnant C57BL/6 mice (NP). Among other gene, we observed that expression of Il1rl1 (ST2) was significantly up-regulated in B cells from P compared to NP mice. Further flow cytometry analysis showed that expression of ST2 was increased on total splenic B cells from P compared to NP mice and it was predominantly expressed on $B220^{low}CD23^{neg}$ B1 B cell subset.
A kinetic analysis depicted that levels of splenic ST2-expressing B1 cells were significantly increased at early pregnancy (day 12) and mid-pregnancy (day 14) as compared to NP mice. Additionally, we evaluated the expression of ST2-expressing B cells in a murine model of preterm birth. Interestingly, the levels of ST2-expressing B cells were significantly increased in the spleen of LPS-induced preterm birth mice compared to normal pregnant mice. Bringing all these data together, our results reinforce the idea of IL-33 being a crucial cytokine controlling pregnancy outcome through a mechanism involving ST2 expression in B cells.

P.A5.02.15

Effect of adjuvant on immune responses to an experimental subunit vaccine antigen in sheep

***S. R. Wattegedera**[1], K. Stronach[2], E. Rampacci[3], N. Arteche Villasol[4], R. Tassi[1], J. Thomson[1], M. Rocchi[1], K. Aitchison[1], M. Livingstone[1], D. Longbottom[1], G. Entrican[1];*
[1]Moredun Research Institute, Penicuik, United Kingdom, [2]University of Edinburgh, Edinburgh, United Kingdom, [3]Universita degli Studi di Perugia, Perugia, Italy, [4]Universidad de Leon, Leon, Italy.

Introduction: The desired goal of vaccines to protect against intracellular bacterial infections is usually the induction of cellular T-helper (Th)-1 type immunity, characterised by the production of interferon (IFN)-gamma. This has been particularly challenging for subunit vaccine development in livestock. We have characterised cellular immune responses in sheep to an experimental chlamydial subunit vaccine antigen delivered in three adjuvants.
Materials and Methods: Groups of 35 sheep were immunised with a single inoculation of the experimental vaccine antigen formulated in two water-in-oil adjuvants (Montanide ISA 70 VG, Montanide ISA 61 VG) or saponin-derived QuilA. Peripheral blood mononuclear cells (PBMC) were isolated pre- and post-immunisation and re-stimulated *in vitro* with both the vaccine antigen and whole killed chlamydial elementary bodies (EBs). Recall responses were measured by the presence of cytokines in the PBMC culture supernates, with IFN-gamma as an indicator of Th1-type responses and interleukin (IL)-4 as an indicator of Th2-type responses.
Results: All three adjuvants induced antigen-specific immune responses that could be detected in recall assays to both the experimental vaccine antigen and whole chlamydial EBs. In each case, IFN-gamma was the dominant cytokine in the post-immunisation antigen-specific recall responses, with almost no antigen-specific IL-4 being detected. Mitogen-induced IFN-gamma and IL-4 was consistent pre- and post-immunisation. Of the three adjuvants, Montanide ISA 61 VG induced slightly higher levels of IFN-gamma.
Conclusions: Classical Th1-type responses can be elicited in sheep to a subunit antigen delivered in different adjuvants. The relative efficacy of these adjuvants needs to be determined using infection challenge models.

P.A5.02.16

The chemokine receptor CX3CR1 defines three antigen-experienced CD8 T cell subsets with distinct roles in immune surveillance and homeostasis

***C. Gerlach**[1,2], E. Moseman[2], S. M. Loughhead[2], D. Alvarez[2], A. J. Zwijnenburg[1,2], L. Waanders[2], R. Garg[2], J. C. de la Torre[3], U. von Andrian[2];*
[1]Karolinska Institutet, Stockholm, Sweden, [2]Harvard Medical School, Boston, United States, [3]The Scripps Research Institute, La Jolla, United States.

Infections induce pathogen-specific T cell differentiation into diverse effectors (Teff) that give rise to memory (Tmem) subsets. The cell-fate decisions and lineage relationships that underlie these transitions are poorly understood. Here, we found that the chemokine receptor CX3CR1 identifies three distinct $CD8^+$ Teff and Tmem subsets. Classical central (Tcm) and effector memory (Tem) cells and their corresponding Teff precursors were $CX3CR1^-$ and $CX3CR1^{high}$, respectively. Viral infection also induced a numerically stable $CX3CR1^{int}$ subset that represented ~15% of blood-borne Tmem cells. $CX3CR1^{int}$ Tmem cells underwent more frequent homeostatic divisions than other Tmem subsets and not only self-renewed, but also contributed to the expanding $CX3CR1^-$ Tcm pool. Both Tcm and $CX3CR1^{int}$ cells homed to lymph nodes, but $CX3CR1^{int}$ cells, and not Tem cells, predominantly surveyed peripheral tissues. As $CX3CR1^{int}$ Tmem cells present unique phenotypic, homeostatic, and migratory properties, we designate this subset peripheral memory (Tpm) cells and propose that Tpm cells are chiefly responsible for the global surveillance of non-lymphoid tissues.
This study was supported by a Rubicon fellowship (Netherlands Organization for Scientific Research, NWO) and a postdoctoral fellowship of the Cancer Research Institute Irvington Fellowship Program to CG, NIH T32 Training Grant in Hematology HL07623-20 to EAM, NIH F31 grant CA171339 to SML, NIH T32 grant HL066987 to DA, the Ragon Institute of MGH, MIT and Harvard and NIH/NIAID RO1 AI069259, PO1 AI078897 and PO1 AI112521 to UHvA.

P.A5.02.17

Inducing anti-tumoral immunity with dendritic cell reprogramming

***F. Fiúza Rosa**[1,2,3], C. Pires[1,2,3], I. Kurochkin[4], A. Gomes[3], M. Humbert[5], A. Ferreira[1,2,3], L. Palma[3], K. Shaiv[3], L. Solanas[3], C. Azenha[3], D. Papatsenko[4], O. Schulz[6], S. Hugues[5], C. Reis e Sousa[6], C. Pereira[1,2,3];*
[1]Lund Stem Cell Centre, Lund, Sweden, [2]Wallenberg Centre for Molecular Medicine, Lund University, Lund, Sweden, [3]Centre for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal, [4]Skolkovo Institute of Science and Technology, Moscow, Russian Federation, [5]Department of Pathology and Immunology, University of Geneva Medical School, Geneva, Switzerland, [6]Immunobiology Laboratory, The Francis Crick Institute, London, United Kingdom.

Cell fate reprogramming of adult cells towards pluripotency or unrelated somatic cell-types has been explored in the context of regenerative medicine. Dendritic cells (DCs) are professional antigen presenting cells (APCs) specialized in the recognition, processing and presentation of antigens to T-cells, inducing adaptive immunity. We hypothesized that the unique properties of DCs could be induced in unrelated cell-types, allowing the direct control of immune responses with cell reprogramming. Here, the requirements to induce DCs were investigated using a combinatorial overexpression of Transcription Factors (TFs) in Clec9a-tdTomato mouse fibroblasts. This reporter system specifically marks the conventional DC lineage. We have identified Pu.1, Irf8 and Batf3 as sufficient and necessary to induce Clec9a reporter activation and establish DC morphology and phenotype in fibroblasts. Induced DCs (iDCs) express DC type 1 (cDC1) surface phenotype and transcriptional program, with features of interferon-induced maturation. Functionally, iDCs engulf proteins, dead cells and upon stimulation of toll-like receptors, secrete inflammatory cytokines. iDCs capture, process and present antigens to CD4+ T and remarkably, CD8+ T cells, a hallmark of cross-presenting DCs. Strikingly, vaccination of syngeneic tumor mouse models with iDCs inhibited tumor growth. Finally, transduced human fibroblasts acquire DC morphology, DC phenotype and competence to engulf proteins and dead cells. Hence, we provide evidence that antigen presentation and cross-presentation can be dynamically programmed by a small combination of TFs. These findings provide insights into cDC1 specification and a platform for future development of cancer immunotherapies based on cell reprogramming.

P.A5.02.18

TLR2-mediated inflammatory responses and their impact on the course of dengue virus infection

*A. Aguilar Briseno[1], V. Upasani[1,2], B. M. ter Ellen[1], J. Moser[3], P. Dussart[2], T. Cantaert[2], J. M. Smit[1], **I. A. Rodenhuis-Zybert**[1];*
[1]Department of Medical Microbiology, University of Groningen and University Medical Center Groningen, Groningen, Netherlands, [2]Institut Pasteur du Cambodge, International Network of Pasteur Institutes, Phnom Penh, Cambodia, [3]Departments of Pathology & Medical Biology and Critical Care, University of Groningen and University Medical Center Groningen, Groningen, Netherlands.

Clinical manifestations of dengue virus (DENV) infections range from a flu-like to a severe disease hallmarked by increased vascular permeability and/or plasma leakage. Exacerbated inflammation precedes severe disease; however, its underlying mechanisms are only partially understood. Consequently, there are no methods to predict or block DENV pathogenesis. Toll-like receptors (TLR) play a crucial role in the initiation of inflammation and containment of infections. Yet, prolonged activation of TLRs exacerbates inflammation, which ultimately leads to (vascular) immunopathology. We and others have shown increased expression of TLR2 on monocytes of DENV infected patients compared to healthy controls during the acute phase of infection. Here, we combined *ex vivo*and *in vitro*analyses to identify the relevance of TLR2 expression on peripheral blood mononuclear cells in DENV pathogenesis. We found that TLR2 expression on $CD14^{++}CD16^{-}$classical monocytes isolated during acute DENV infection correlated with disease severity. *In vitro*, blocking of TLR2 prior to DENV infection abolished inflammatory responses mediated by NF-kappaβresulting in diminished intracellular cytokine production and attenuated human endothelial cells activation. Furthermore, blocking the engagement of TLR2 and CD14 but not that of TLR1/6 significantly reduced infected cell-mass, suggesting that DENV usurps TLR2 and CD14 to establish infection. Consistent with these findings, in patients, DENV infection was evident primarily in $CD14^{++}CD16^{-}$monocytes, which correlated with the development of severe disease.Conclusively, our data reveal the fundamental role of TLR2 as a regulator DENV-induced inflammation and immunopathology. Pharmacological targeting of the TLR2 axis could form a strategy for mitigating the pathogenesis of severe disease.This study was supported by NWO-VENI grant and the Institut Pasteur International Network.

P.A5.03 Initiation of immune responses - Part 3

P.A5.03.01

HIV-1 hijacks the complement system to escape degradation and promote viral dissemination by human Langerhans cells

***M. Bermejo Jambrina**[1], B. Nijmeijer[2], D. Wilflingseder[1], T. Geijtenbeek[2];*
[1]Department of Hygiene and Medical microbiology, Medical University of Innsbruck, Innsbruck, Austria, Innsbruck, Austria, [2]Department of Experimental Immunology, Amsterdam Infection and Immunity Institute, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands.

The role of complement in HIV-1 susceptibility remains unclear. *In vivo*, HIV-1 spontaneously activates complement in semen, plasma and at mucosal surfaces and is therefore coated after viral entry. However, whether this is involved in viral transmission is unknown. Langerhans cells (LCs) reside in mucosal tissue and are the first cells that encounter HIV-1 during sexual contact. LCs are able to limit dissemination by degrading HIV-1 via langerin-induced autophagic processes, preventing HIV-1 infection of LCs and transmission to T cells. We set out to investigate the role of complement in sexual transmission of HIV-1 using isolated human LCs and the *ex vivo* skin transmission model. Strikingly, complement-opsonized- HIV-1, in contrast to non-opsonized HIV-1, efficiently infected LCs, *in vitro* and *ex vivo*. Moreover, complement-opsonized-HIV-1 was efficiently transmitted to target T cells by LCs in the *ex vivo* skin transmission modelInfection and subsequent transmission of LCs were inhibited by blocking CR3 and CR4.
Using isolated activated LCs we observed that complement opsonization increased HIV-1 binding to LCs through CR3 and CR4. Langerin inhibition reduced binding of complement-opsonized-HIV-1 but both CR3 and CR4 were most important for binding. These data suggest that complement opsonization leads to a different routing of HIV-1 in LCs via CR3 and CR4 binding, evading antiviral function of langerin and increasing HIV-1 infection of LCs and subsequent viral dissemination. This study provides novel insight into the importance of complement in HIV-1 susceptibility and might lead to preventative strategies to prevent HIV-1 infection. This work was funded by EFIS.

P.A5.03.02

Unraveling the regulation of Antigen-specific Immunoglobulin Glycosylation

***E. L. de Graaf**[1], R. Visser[1], A. Hipgrave-Ederveen[2], C. Koeleman[2], E. van der Schoot[1], M. Wuhrer[2], G. Vidarsson[1];*
[1]Sanquin Research, Amsterdam, Netherlands, [2]Centre for Proteomics and Metabolomics, LUMC, Leiden, Netherlands.

We have recently found that antibodies formed against platelet and red blood cell antigens to be skewed towards a unique type of N-linked IgG Fc-glycan profile with decreased fucosylation, increased galactosylation and sialylation. The lowered core-fucosylation increases the affinity of the pathogenic antibodies to FcγRIIIa and FcγRIIIb, and hence platelet/RBC destruction. More remarkably, the Fc-glyco profile seems to be stable for years and even decades after immunization. In order to understand how the Ig glycosylation is regulated on the B cell level, we have set up a B cell culture system where we can follow antigen-specific glycosylation in clonally related cells. By following the development of an early memory B cell (i.e. CD27+, IgM+) into a late state memory B cell (CD27+, IgG+) characterized by low and high somatic hypermutation, respectively, we attempt to determine the point of IgG glyco-memory formation. Firstly, antigen-specific B-cell clones from affected donors were isolated, FACS sorted and individually expanded *in vitro*. Thereafter, antibody glycosylation profiles were determined by measuring Fc glycopeptide abundances from each supernatant, using liquid chromatography and mass spectrometry. Subsequently, the acquired B-cell-specific IgG glycoprofiles will be correlated to the clonal relation and mRNA expression of a panel of glycosylating enzymes of each clone. Initial results will be presented, providing more insight in the regulation of antigen-specific antibody glycosylation.

P.A5.03.03

NK-cell mediated ADCC via FcγRIIIa is affected by IgG3 polymorphisms

***S. W. de Taeye**, A. E. Bentlage, S. Lissenberg-Thunnissen, E. L. de Graaf, T. Rispens, G. Vidarsson;*
Sanquin, Amsterdam, Netherlands.

Besides immunoglobulin isotypes and subclasses, polymorphisms in the immunoglobulin gamma heavy chain gene form another layer of variation to the humoral antibody response (IgG allotypes). From the four IgG subclasses (IgG1-4), most variation has been found in IgG3. Interestingly, several allotypes have been linked with susceptibility to various infectious diseases or auto-immune diseases. To study the influence of the polymorphisms on IgG effector functions, we produced all the described allotypes (27 allotypes, anti-RhD specificity) and subsequently assessed Fc gamma receptor (FcγR) binding with surface plasmon resonance (SPR). When we compared IgG3 allotypes, we observed small differences in binding to Fc gamma receptors FcγRIIa and FcγRIIIa. Most prominently, allotype IGHG3*18 and IGHG3*19 bound less well to FcγRIIIa than all the other IgG3 allotypes. The three-fold lower affinity of these allotypes for FcγRIIIa directly correlated with a reduced capacity to induce ADCC. Allotype IGHG3*18 and IGHG3*19 express a unique tryptophan at position 292 in the CH2 domain instead of an arginine, which is not found in the other IgG subclasses. Since residue 292 is not directly involved in binding to FcγRIIIa, we hypothesize that the variation at position 292 alters the conformation of the loop that interacts with FcγRIIIa, which is a likely possibility based on available structural data. Future experiments are necessary to understand the interaction of IgG allotypes with FcγR, but also the neonatal Fc-receptor, and complement. These experiments should give new insights how these allotypes may possibly be linked with susceptibility to infectious diseases, allo- or auto-immune diseases.

P.A5.03.04

T cells enhance direct infection of NK cells by viruses, leading to enhanced effector functions

*M. Lambregts, N. Swaans, M. Emmelot, E. A. van Erp, D. van Baarle, **J. de Wit**;*
National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands.

Natural Killer (NK) cells are important in virus-infections and were originally believed to mainly recognize and eliminate infected cells through cytotoxicity. Increasing evidence now illuminates the immune modulatory functions of NK cells, via (pro-)inflammatory cytokines or intercellular interactions, underlining the complexity of NK-cell crosstalk with both innate and adaptive immune cells.
Previously, we found a significant contribution of NK cell to the IFN-γ response against mumps virus (MuV). Here we sought to investigate the direct effects of virus-interactions with NK cells, and the crosstalk with other immune cells herein.
Incubation with live MuV resulted in a substantial infection of isolated human NK cells (up to 45%). NK cell infection was even further enhanced in presence of other PBMCs (up to 80%), implying crosstalk with other immune cells. Indeed, depletion of T cells reduced the infection of NK cells. The T-cell mediated enhanced infection of NK cells was also observed for other viruses (including measles and respiratory syncytial virus), suggesting a broader phenomenon. Following infection, NK cells were strongly activated and displayed enhanced effector functions, as shown by IFN-γ production and release of granzymes and perforin.
The implication of the enhanced effector functions on the NK-mediated immune modulation remains to be elucidated.

P.A5.03.05

Caspase 1 activity impairs CD8⁺ T cells responses in coronavirus induced hepatitis

M. Duhalde Vega[1,2,3], *M. Jeldres*[1,3], *M. Hill*[1,4,3];
[1]*Laboratory of Immunoregulation and Inflammation. Institut Pasteur de Montevideo., Montevideo, Uruguay,* [2]*Institute of Biochemistry and Biophysics (IQUIFIB, UBA-CONICET), Buenos Aires, Argentina,* [3]*Centre for Translational Immunology. FOCIS Centre of Excellence. Montevideo Faculty of Medicine, Institut Pasteur de Montevideo., Montevideo, Uruguay,* [4]*Immunobiology Department. Faculty of Medicine. University of the Republic, Montevideo, Uruguay.*

Introduction: The inflammasomes play a crucial role in the immune response to viral infection. Activation of inflammasome triggers the cleavage of caspase-1 and maturation of IL1β, a critical step for an efficient infection control. The positive relation between IL1β release and adaptive immunity has been exhaustively described, but hepatitis seems to be negatively influenced by the inflammasome. In HCV patients, IL1β level is augmented and it has been associated with immunopathology and viral load. Therefore, the aim of this work was to further analyze the role of the Caspase-1-IL1β axis in the initiation of adaptive immune responses. Methods: Cells and animals were infected with Mouse Hepatitis Virus strain A59 (MHV). We use WT and Caspase 1-deficient mice (Casp1$^{-/-}$). Results: BMDCs derived from Casp1$^{-/-}$ and wt mice were infected with MHV and inflammasome activation was analyzed. Data have shown that MHV infection induced IL1β release on WT mice, but not on Casp1$^{-/-}$BMDCs. Then, in vivo studies have shown that Casp1 deficiency ameliorates MHV-induced hepatitis. Survival rates revealed that Casp1$^{-/-}$ mice are resistant to MHV infection, while only 25% of wt mice survive at 20dpi. In accordance, liver from Casp1$^{-/-}$ mice showed reduced levels of MHV-RNA and augmented liver CD8-Tcells infiltration. Moreover, Casp1$^{-/-}$ mice have elevated number of MHV-specific CD8-T-cells and higher expression of CD107a marker. Finally, in vivo CTL activity assay confirmed that Casp1$^{-/-}$ mice have higher MHV-specific CTL activity than wt mice. Conclusion: We found that Caspase 1 activity is crucial in the modulation of CD8-T-cell response to coronavirus-induced hepatitis.

P.A5.03.06

A phosphatidylinositol 4, 5-biphosphate (PIP2) metabolism-derived amplification loop fuels the sustained initiation of B cell activation

W. LIU*, C. Xu;*
TSINGHUA UNIVERSITY, BEIJING, China.

Lymphocytes have evolved sophisticated signaling amplification mechanisms to efficiently activate downstream signaling following detection of rare ligands in their microenvironment. B cell receptor microscopic clusters (BCR microclusters) are assembled on the plasma membrane and recruit signaling molecules for the initiation of lymphocyte signaling after antigen binding. Here, we identified a signaling amplification loop derived from phosphatidylinositol 4, 5-biphosphate (PIP2) for the sustained B cell activation. Upon antigen recognition, PIP2 was depleted by phospholipase Cγ2 (PLCγ2) within the BCR microclusters and was regenerated by phosphatidic acid (PA)-dependent type I phosphatidylinositol 4-phosphate 5-kinase (PIP5K) outside of the BCR microclusters. The hydrolysis of PIP2 inside of the BCR microclusters induced a positive feedback mechanism for its synthesis outside of the BCR microclusters. The falling gradient of PIP2 across the boundary of BCR microclusters was important for the efficient formation of BCR microclusters. Our results identified a PIP2 derived amplification loop that fuels the sustained initiation of B cell activation.

P.A5.03.07

Anti-domain 1 beta2-glycoprotein I antibodies induce activation of monocytes and NK cells, and provoke prothrombotic settings

A. Martirosyan[1], *T. Papajik*[2], *S. Margaryan*[1,3], *Z. Mikulkova*[3], *L. Slavik*[2], *J. Ulehlova*[2], *E. Kriegova*[3], *G. Manukyan*[1,3];
[1]*Laboratory of Molecular and Cellular Immunology, Institute of Molecular Biology NAS RA, Yerevan, Armenia,* [2]*Department of Hemato-oncology, Faculty of Medicine and Dentistry, Palacky University Olomouc and Faculty Hospital, Olomouc, Czech Republic,* [3]*Department of Immunology, Faculty of Medicine and Dentistry, Palacky University Olomouc and Faculty Hospital, Olomouc, Czech Republic.*

Introduction: It has been suggested that antibodies against domain 1 (D1) of β2-glycoprotein I (β2GPI) have clinical relevance in antiphospholipid syndrome (APS) patients, and strongly correlate with thrombosis and pregnancy complications. The direct influence of anti-D1 β2GPI on activation and pro-thrombotic activity of immune cells has not been studied yet. We aimed to determine the influence of anti-D1 β2GPI IgG antibodies on immune cells *in vitro*. Methods: For this, peripheral blood mononuclear cells from 11 healthy individuals were incubated (for 24 hours) with: 1) pooled plasma (n=6) derived from APS patients contained anticardiolipin antibodies (aCL), lupus anticoagulant (LA), anti-β2GPI and anti-D1 β2GPI; 2) pooled plasma (n=6) derived from APS patients contained aCL, LA, anti-β2GPI, and negative for anti-D1 β2GPI; 3) seronegative (negative for antiphospholipid antibodies) pooled plasma (n=6). Results: The presence of anti-D1 β2GPI markedly induced a proinflammatory phenotype of monocytes and NK cells in comparison with the cells cultured with anti-D1 β2GPI-negative and seronegative plasma. Particularly, anti-D1 β2GPI significantly increased % and MFI of CD142 (tissue factor, TF), HLA-DR and CD11 on healthy monocytes. A greater percentage of CD69+ NK cells was found upon cultivation with anti-D1 β2GPI+ plasma. Expression of IgG receptor FcγRIIIa (CD16) on both monocytes and NK cells was down-regulated by anti-D1 β2GPI + plasma. Conclusion: Taking together, for the first time, we demonstrated strong activation of monocytes and NK cells exposed to anti-D1 β2GPI. Prominently, anti-D1 β2GPI induced substantial increase in expression of monocytic TF favoring an initiation of thrombus formation. Grant support: IGA UP_2018_016

P.A5.03.08

Antigen-specific activation of murine B lymphocytes *in vitro*

S. Michelchen*, K. Hanack;*
University of Potsdam, Department of Biochemistry and Biology, Potsdam, Germany.

Introduction: The generation of monoclonal antibodies by hybridoma technology is currently performed by immunizing animals such as mice with the desired antigen. The immune reaction takes place *in vivo* without any opportunities to intervene. The transfer of antigen-specific immune responses to *in vitro* conditions would allow a monitoring of these processes in a defined culture environment. For this, a simplified set-up was established in which only B lymphocytes were cultured with supplements mimicking the *in vivo* conditions of antigen-specific activation.
Methods: Murine splenic B lymphocytes from naive mice were isolated by magnetic cell sorting and cultivated *in vitro* with different combinations of antigen, αCD40-antibody, LPS, IL4 and IL7. As antigen a viral protein from the hamster polyomavirus capsid (VP1) was used. Antibody responses were determined by ELISA and Western Blot. B cell phenotypes were investigated by flow cytometry. Positive cultures were used to generate stable antibody producing hybridomas.
Results: VP1-specific antibody responses in *in vitro* cultures could be detected from day 3 on with specific IgM-responses. At day 9 we detected specific IgG-antibodies in cultures stimulated with antigen, αCD40-antibody and IL4. By fusion of antigen-specific B lymphocytes we were able to create IgM- and IgG-producing hybridoma cell lines.
Conclusions: Specific B cell activation *in vitro* was successfully shown within 10 days of *in vitro* culture. Combined with hybridoma technology, this method could be a powerful future approach in the field of antibody production.

P.A5.03.09

CD27-mediated stimulation of human CD4⁺ T-cells leads to a reduction in active HIV-production

S. Nüssing[1], *R. M. van der Sluis*[2], *E. B. Clemens*[1], *T. H. Nguyen*[1], *J. L. Anderson*[2], *F. Luciani*[3], *A. Al-Shamkhani*[4], *S. R. Lewin*[2], *K. Kedzierska*[1];
[1]*Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Australia,* [2]*The Peter Doherty Institute for Infection and Immunity, The University of Melbourne and Royal Melbourne Hospital, Melbourne, Australia,* [3]*School of Medical Sciences, UNSW, Sydney, Australia,* [4]*Cancer Sciences Unit, Faculty of Medicine, University of Southampton, Southampton, United Kingdom.*

Despite recent advances in antiretroviral-therapy (ART) for HIV-infection, life-long treatment is required as the virus persists in latently infected cells. Latently infected cells contain integrated virus but can escape immune-recognition due to minimal transcription. Innovative approaches eliminate latency by activating HIV-transcription to stimulate viral-production, which can lead to virus-mediated cytotoxicity or immune recognition of infected cells and ultimate elimination of HIV. We hypothesized that stimulation of CD4⁺ T-cells through the CD27/CD70-pathway would be a potent driver for HIV-transcription. Stimulation of naïve CD4⁺ T-cells with anti-CD3 antibody and CD70 ligand resulted in their potent activation, increased proliferation, granzyme B and perforin production, as compared to conventional anti-CD3/anti-CD28 antibody co-stimulation. In an *in vitro* model of HIV-infection of CD4⁺ T-cells, co-cultured with monocytes to facilitate infection of resting cells, stimulation with anti-CD3/CD70 compared to anti-CD3/anti-CD28 resulted in a lower frequency of virus activation. Moreover, in a reciprocal experiment with resting CD4⁺ T-cells stimulated *in vitro* via the CD27- or CD28-pathway prior to infection, strikingly, we found less virus-positive CD4⁺ T-cells under CD27-stimulating conditions, suggesting these cells were refractory to HIV-infection. We then stimulated CD4⁺ T-cells collected from HIV+ individuals on ART *ex vivo* and quantified cell-associated unspliced HIV-RNA by qPCR. Further, we performed single-cell RNA-sequencing on CD28- or CD27-activated cells to delineate gene expression profiles associated with these signaling pathways and to understand their differing effect on HIV-infection. Overall, our studies reveal novel insights into the potent effects of CD27/CD70 stimulation on CD4⁺ T-cells and containment of viral infection in HIV+ patients.

P.A5.03.10

Cell adaptation of monoclonal antibodies produced by Chinese hamster Ovary (CHO) cell to grown in serum-free medium

***W. Puangmanee**;*
Faculty of Tropical Medicine, Bangkok, Thailand.

Wilarat Puangmanee[1], Pongrama Ramasoota[1], Pannamthip Pitaksajjakul[1]
1 Department of Social and Environmental Medicine, Faculty of Tropical Medicine, Mahidol University

Monoclonal antibody (MAb) is one of the most widely used substance for biopharmaceuticals and therapeutics for viral disease, especially dengue virus which is one of important re-emerging virus may causing life-threatening disease around the world. MAb can be produce using immunoglobulin G (IgG) gene cloning and expression in Mammalian Chinese Hamster Ovary (CHO) cell. Therefore, in vast value, it is necessary to produce in efficient large-scale production for control quality of MAbs. From our successful generation, human monoclonal antibodies (HuMAbs) were produced by fusion between myeloma cells and B-cells using hybridoma technology. These HuMAbs showed cross-neutralizing activity to dengue virus 4 serotypes. The recombinant IgG form of those HuMAbs showed prefer neutralizing (NT) activity.
For further characterization and standardization of HuMAbs, generaton of stable CHO cells for HuMAb production is required. In this study, plasmid of HC and LC were transfected to CHO cell using lipofectamine 2000. Then, stable CHO cells were selected by two antibiotics, puromycin and hygromycin. Then, high producer CHO cell was selected by flow cytometer. The single clones were subcloned by limiting dilution. Single clone that showed high production of HuMAb were screened by IgG quantitation ELISA. Stability of stable CHO cell was tested. The clones that showed high stability in antibody production were re-cloned and proceeded for suspension cell adaptation and production in serum-free medium for further scale-up.
Keywords: Human monoclonal antibody, Mammalian cell, Neutralization activity

P.A5.03.11

PD1 ligand regulation during viral infection: Primus inter pares

***M. Raftery**, J. Hofmann, G. Schönrich;*
Institut für Virologie, Berlin, Germany.

Enhancement of the immune response to tumours and infections by blocking inhibitory co-stimulation has become an established success. In particular blocking of the CD28 family member PD1 on T cells and its ligands, PD-L1 and PD-L2, has proven therapeutically effective. The regulation of these ligands, however, has not yet been fully elucidated although cell-surface expression is known to be induced by interferons.In order to better quantify the upregulation of PD-L1 and PD-L2 in response to stimuli we exposed primary cells (fibroblasts, endothelial cells, dendritic cells and PBMC) to different interferons and PAMPs as well as to active viral infections. Cells were analysed by flow cytometry and functional assays.Comparison between Type I, II and III interferons showed a surprising divergence between otherwise similar cytokines, ranging from no induction (III) to strong (II). Similarly, some viral PAMPs showed strong induction whereas others such as RIG-I appeared to be ineffective. Viral infection in general rapidly induced PDL expression. This might argue for a role in the innate immune response to viral infection in addition to downregulation of the adaptive immune response as has been previously demonstrated. In agreement with this was the association of bystander activation with PDL expression in response to primary infection of PBMC. We propose that the PD1 system has a role to play in innate as well as adaptive immunity.

P.A5.03.12

Alum induces rapid NADPH-oxidase independent NET release in human neutrophils

***M. Reithofer**[1], D. Polak[1], C. Kitzmüller[2], G. Greiner[3], B. Bohle[2], B. Jahn-Schmid[1];*
[1]Institute for Pathophysiology and Allergy Research, MCCA PhD Programme, Vienna, Austria, [2]Institute for Pathophysiology and Allergy Research,, Vienna, Austria, [3]Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria, Vienna, Austria.

Alum is the most widely used adjuvant, though the mechanism behind its adjuvanticity is not totally solved. In mice, host-derived DNA has been reported to be involved in the adjuvant effect of alum. Neutrophils are the first cells at the site of injection and in response to strong or particulate stimuli neutrophils have the ability to simultaneously release extracellular DNA and granular material, so-called neutrophil extracellular traps (NETs) which are able to trap and kill microbes.
Here, we investigated alum-induced NET-formation in human neutrophils and its underlying pathway. Neutrophils were stimulated with alum or PMA and ionomycin as positive controls. Strong NET-formation was induced by all stimuli as visualized by fluorescence microscopy showing co-localization of extracellular DNA and different granular proteins. In addition, alum-induced neutrophil elastase activity was found in supernatants. Inhibition of downstream signalling molecules by using a plate-reader assay to quantify released DNA were performed, to reveal the pathway underlying NET-formation. Ionomycin and alum-induced mitochondrial reactive oxygen species (mROS), whereas PMA triggered cytoplasmatic NADPH oxidase-dependent ROS. Alum induced rapid DNA-release similar to ionomycin and dependent on phagocytosis, extracellular calcium and NFκB signalling. Furthermore, a significant dependence on necroptosis signalling similar to crystal-induced NET release was found. During the process of NET formation, increased glycolysis, as well as mitochondrial respiration was observed.
Together, alum potently induces a rapid mROS dependent NET-release in human neutrophils *in vitro,* utilizing energy from glycolysis and mitochondrial respiration. These NETs may represent danger-associated molecular patterns involved in the initial immune response to alum-adjuvated vaccines.

P.A5.03.13

Distinct roles for Btk in the formation of the B cell immune synapse

***S. Roman-Garcia**[1], S. R. Gardeta[1], S. V. Merino-Cortes[1], M. J. de Bruijn[2], R. W. Hendriks[2], Y. R. Carrasco[1];*
[1]CSIC, Madrid, Spain, [2]Erasmus University Medical Center, Rotterdam, Netherlands.

Bruton's tyrosine kinase (Btk) has a key role in the signaling pathways of receptors essential for the B lymphocyte response. Given its implication in B cell-related immunodeficiencies, leukemias/lymphomas and autoimmunity, Btk is studied intensely and is a target for therapy. Here we report distinct roles for Btk in antigen-triggered immune synapse (IS) formation of mouse primary B cells. Btk recruitment to the plasma membrane regulates the B cell ability to trigger IS formation as well as its appropriate molecular assembly; Btk shuttling/scaffold activities seem more relevant than the kinase function on that. Btk-kinase activity controls antigen accumulation at the IS through the PLCγ2/Ca^{2+} axis. Impaired Btk membrane-recruitment or kinase function likewise alters antigen-triggered microtubule-organizing center (MTOC) polarization to the IS, B cell activation and proliferation. We also show that, for B cell function, IS architecture is as important as the quantity of antigen that accumulates at the synapse.

P.A5.03.14

IL-10 competence and production in murine B cells: find the differences!

***S. Tonon**[1], F. Mion[1], J. Dong[2], H. Chang[2], M. Colombo[3], E. Dalla[1], A. Radbruch[2], C. E. Pucillo[1];*
[1]University of Udine, Udine, Italy, [2]German Rheumatism Research Center (DRFZ), Berlin, Germany, [3]Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy.

Under physiological conditions the immune system is maintained under homeostasis thanks to the balance between the regulatory and the effector compartment. Regulatory cells can be detected among several immune populations: B cells with regulatory functions have been described, but still no transcription factor to identify them has been discovered. Our working hypothesis is that we can distinguish between IL-10-competent B cells and IL-10-producing B cells, where the first class is ready for IL-10 production immediately after stimulation, while all the others are instructed by the surrounding environment. After 48 hours of stimulation IL-10 production can be induced by infective stimuli, such as LPS or CpG, but not by immune-mediated like through CD40. Very interestingly, if cells that are pre-stimulated through CD40 receive a second stimulation they start transcribing *il10*: these cells are competent for IL-10 production. Of note, the same concept can be applied in the *ex vivo* situation. Indeed, among total murine splenic B cells stimulated for 5 hours with LPS, PMA and ionomycin, only the 2-3% is able to immediately produce IL-10 and these are genuine IL-10 competent B cells. We set up a method to isolate them at very high purity taking advantage of an IL-10 secretion assay combined with FACS-sorting. On these two populations we performed several analysis, trying to understand which are the mechanisms at the basis of IL-10 production. We first analysed the timing of IL-10 production and then compared the transcriptomic signature of IL-10-competent and non-competent B cells with the public database ImmGen.

P.A5.03.15

Activation of the cGAS-STING pathway by chitosan requires the engagement of Dectin-1

J. L. Turley;
Trinity College Dublin, Dublin 2, Ireland.

The cationic polysaccharide, chitosan is an effective adjuvant that induces humoral immunity and Th1 cell responses following vaccination by injection or mucosal routes, supporting its application as an alternative to alum for vaccines that promote cell-mediated immunity.
We previously reported that chitosan promotes dendritic cell (DC) maturation by inducing type I interferons and enhances antigen-specific Th1 responses in a type I IFN receptor-dependent manner. We have shown this response is dependent on C type lectin (CTL) Dectin-1 sensing, mitochondrial disruption, subsequent mtDNA release and the cGAS-STING sensing pathway. Here we propose that chitosan binds to the Dectin-1 receptor leading to the activation of phospholipase Cγ-2 (PLCγ-2) and the efflux of calcium from the endoplasmic reticulum (ER). A localised actin polymerisation event then brings the ER and mitochondria into close proximity, allowing a disproportionate amount of calcium to flow into the mitochondria. High calcium levels in the mitochondria result in increased ROS production and mitochondrial depolarisation, likely contributing to the subsequent release of mtDNA. Our data indicate that the immunomodulatory properties of chitosan result from this calcium-driven ROS production. This work provides evidence for the first time of a link between CTL receptors and intracellular nucleic acid receptors leading to dendritic cell maturation and enhanced cellular immunity.

P.A5.03.16

Selective recruitment of CD8⁺ T cells against a novel 12-mer A*68:01-restricted influenza peptide reflects the importance of HLA and TCR profiles

*E. B. Clemens[1], **C. E. van de Sandt**[1,2], E. Grant[1], S. Gras[3], J. Rossjohn[3], W. Chen[4], K. Kedzierska[1];*
[1]University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Australia, [2]Sanquin Blood Supply Foundation, Amsterdam, Netherlands, [3]Monash University, Melbourne, Australia, [4]La Trobe University, Melbourne, Australia.

Influenza A viruses are responsible for seasonal epidemics and sporadic pandemics that result in significant health, social and economic costs worldwide. In contrast to strain-specific antibodies, immunity provided by CD8⁺ T cells is long-lasting and cross-strain specific, making it an attractive target for novel universal one-shot vaccine strategies. We recently identified a novel 12-mer peptide from influenza A virus NP protein ($NP_{145-156}$) restricted by HLA-A*68:01. To determine the potential for NP_{145}-specific CTL to contribute to anti-influenza immunity, we dissected the characteristics of this response in A*68:01⁺ individuals (0-25% allele frequency, depending on ethnicity). We observed NP_{145}-specific responses in ~50% of individuals, ranging from immunodominant to subdominant in magnitude. Remarkably, individuals who did not respond to this epitope nevertheless contained populations of naïve NP_{145}-specific CD8⁺ T cells, suggesting that this precursor population is present but not effectively recruited during infection.
Crystal structure analysis of the A*68:01-NP_{145} complex showed that the central region of the NP_{145} peptide is highly flexible and may present a difficult target for TCR recognition, especially as A*68:01 does not ligate CD8 for enhancement of TCR-pMHC binding. Single-cell multiplex RT-PCR analysis of TCRαβ heterodimers revealed that recruitment of high affinity NP_{145}-specific responses was dependent on expression of distinct TCRαβ signatures characterized by long CDR3α and -β loops. Our data highlight the role of individual HLA profiles and intrinsic CD8⁺ T cell quality in determining recruitment of effective epitope-specific responses during infection.

P.A5.03.17

Bystander T-cells support clonal T-cell activation by controlling the release of dendritic cell-derived immune-stimulatory vesicles.

*M. Lindenbergh[1], D. Koerhuis[1], T. Driedonks[1], R. Wubbolts[1], W. Stoorvogel[1], **M. Boes**[2];*
[1]Utrecht University, Utrecht, Netherlands, [2]UMC-Utrecht, Utrecht, Netherlands.

Extracellular vesicles (EVs) that are released by immune cells are studied intensively for their functions in immune regulation and are scrutinized for their potential in human immunotherapy, for example against cancer. In our search for signals that stimulate the release of functional EVs by dendritic cells (DC) we co-cultured human monocyte-derived DC (moDC) with fixed autologous T-cells. LPS-activated moDC changed their morphological characteristics in response to contact with activated bystander T-cells, while non-activated bystander T-cells had no effect. Exposure of moDC to activated bystander T-cells stimulated the release of moDC-derived EV-associated proteins, including CD9, CD63, CD81, HLA class I, and ICAM-1, although these effects were highly variable between donors, and significant increases could be established only for CD63 and ICAM-1. The release of small RNA species was strongly increased upon interaction with activated bystander T-cells, specifically miR155a, known as a central modulator of T-cell responses, was highly increased in EVs from moDC. Functionally, we observed that EVs from moDC licenced by activated bystander T-cells displayed an enhanced capacity for antigen-specific T-cell activation. Taken together, these results suggest that non-cognate interactions between DC and bystander T-cells can modulate third party antigen-specific T-cell responses via EVs.

P.A5.03.18

Leukocyte iRhom2 regulates basal cardiac function

***S. L. M. Walker**, J. Sanchez, A. Gutierrez Del Arroyo, G. Ackland;*
William Harvey Research Institute, London, United Kingdom.

Introduction: The heart is an immunologically active site, even under basal conditions. Cardio-immune cross-talk regulates myocardial structure and function in basal and pathological conditions. Signaling via TNFR2 may play a cardioprotective role, implicating tumour necrosis factor alpha (TNF-a) in the pathogenesis of cardiac failure. Here, we examined the impact of ADAM-17 sheddase activity on cardiac function using iRhom2 deficient mice in whom only myeloid cells are ADAM-17 deficient, and therefore incapable of shedding TNF-a and CD62L.
Methods: Wild-type and iRhom2 deficient mice of either gender (age 8-12 weeks) underwent echocardiography (Vevo3100, VisualSonics) to measure cardiac physiologic function. Peripheral and cardiac-resident leukocytes were phenotyped by flow cytometry. Molecular markers of cardiac stress (ANP; atrial natriuretic protein) and mitophagy (parkin) were quantified using RT-PCR and Western Blot.
Results: We confirmed that cardiomyocytes express TACE in iRhom2-deficient mice. Peripheral leukocytes, including neutrophils, from iRhom2-knockout mice failed to shed CD62L in response to sterile inflammation (MFI CD62L: KO 7530±3221 Wt 1128±1668; p<0.001; KO n=17 Wt n=12). Constitutively, iRhom2-deficient mice had higher cardiac output at baseline (KO 23.13±1.93 mL/min, Wt 18.05±3.33 mL/min; p<0.001; KO n=11, WT n=9). ANP mRNA was elevated in ventricular tissue of iRhom2-knockout mice ($2^{\Delta CT}$: KO 1.04±0.52 Wt 0.42±0.01; p<0.005; KO n=5 WT n=7), as well as parkin protein levels (Densitometry: KO 0.94±0.97 Wt 0.11±0.10; p<0.05; KO n=5 Wt n=5).
Conclusions: The inability of myeloid cells in iRhom2 deficient mice to cleave CD62L and generate TNF-a is associated with constitutive cardiac injury. This data supports the hypothesis that leukocytes are pivotal in influencing basal cardiac function.

P.A5.03.19

HSPCs prevent chronic stress-induced immune suppression

***D. Yin**[1], H. Zhang[1], Y. Z. Caudle[1], A. Qin[2];*
[1]ETSU College of Medicine, Johnson City, United States, [2]Xiangya School of Pharmaceutical Sciences, Central South University, Changsha, China.

Introduction. Chronic stress has been demonstrated to exert a significant suppressive effect on immune functions. Hematopoietic stem progenitor cells (HSPCs) significantly modulate the immune system. However, the mechanisms responsible for this phenomenon remain to be elucidated. **Methods.** BALB/c male mice were placed in a 50-ml conical centrifuge tube with multiple punctures to establish a chronic restraint stress model. Alterations of regulatory T cells (Tregs) and cell sorting of Tregs for suppressive function assay were investigated. The serum levels of IL-10, IL-12, IL-1β,-10, TGF-β1 was assessed. The expression of STAT4, IL-12Rβ1, Foxp3, CTLA-4, and GITR were determined. **Results**. Administration of HSPCs prevent expansion and alleviate the suppressive activity of Tregs during chronic stress. Moreover, we found this protective effect is dependent on IL-12. IL-12/STAT4 signal provides a critical role against the expansion of Tregs following chronic stress. When neutralizing antibody against IL-12 or treatment with selective STAT4 inhibitor was co-administered with HSPCs in chronic stressed mice, the protective effect of HSPCs was abrogated. Furthermore, HSPCs administration promotes IL-12 production by splenocytes in chronic stressed mice. **Conclusions.** HSPCs prevent chronic stress-induced Tregs expansion in an IL-12/STAT4 dependent manner. Our studies suggest that HPSCs might offer a novel therapeutic strategy against the deleterious effects of chronic stress on the immunosuppression. This work was supported in part by grant NIGM114716.

P.A5.03.20

Dysfunctional proteolysis of CD74 by SPPL2a provokes a humoral response towards CD74 that is specific for ankylosing spondylitis and rheumatoid arthritis

*T. van Kempen[1], E. Leijten[1], M. Lindenbergh[2], M. Olde Nordkamp[1], C. Driessen[3], R. Lebbink[1], M. Wenink[1], N. Baerlecken[4], T. Witte[4], T. Radstake[1], **M. Boes**[1];*
[1]UMC-Utrecht, Utrecht, Netherlands, [2]Utrecht University, Utrecht, Netherlands, [3]Cantonal Hospital St. Gallen, St. Gallen, Switzerland, [4]Medical University Hannover, Hannover, Germany.

Ankylosing spondylitis (AS) is associated with autoantibody production to Class II MHC-associated invariant chain peptide, CD74/CLIP. Mechanisms contributing to this antigen-specific autoimmunity are unknown. We addressed the cause for production of CD74/CLIP-specific antibodies, and observed that AS and rheumatoid arthritis (RA) patient monocytes have compromised activity of the CD74 processing enzyme signal peptide peptidase-like 2A (SPPL2a) and, consequently retained CD74-p8 N-terminal fragments in endosomal compartments. Using THP-1 cells lacking SPPL2a, we reveal a role for SPPL2a in endosomal architecture and surface-directed trafficking of HLA class II, full-length CD74 and CD74-p8. In patient monocytes, we confirm contraction of endosomal size and increase in surface-display of HLA class II and full-length CD74. Moreover, most patients with CD74-p8 accumulation have presence of IgA anti-CD74/CLIP antibodies in serum. Thus, our findings support that SPPL2a dysfunction underscores anti-CD74/CLIP autoantibody formation in AS and RA, through expediting increased display of CD74 self-antigen at the cell surface.

P.A5.04 Initiation of immune responses - Part 4

P.A5.04.01
Transcriptional regulation of the IL-2 gene through Tip60 acetyltransferase binding in the ARRE-2 enhancer element

I. Aggeletopoulou, *I. Panagoulias, F. Karagiannis, P. Davoulou, T. Georgakopoulos, A. Mouzaki;*
Laboratory of Immunohematology, Division of Hematology, Department of Internal Medicine, Faculty of Medicine, University of Patras, Patras, Greece.

Introduction: Acetyltransferase Tip60 regulates gene transcription by interacting with promoter binding factors. Our recent publication has shown that IL-2 expression is blocked by a transcriptional silencer, Ets-2, that binds to the ARRE-2 element of the IL-2 promoter without physical interaction with NFAT-2, which binds to the same element promoting IL-2 activation. In this work, we studied the role of TIP60 on the IL-2 regulation through its possible interactions with Ets-2 and NFAT-2. Methods: Tip60, Ets-2 and NFAT-2 interactions were investigated by co-immunoprecipitation experiments in Jurkat cells in the absence (CM) or presence of the mitogens phorbol myristate acetate and ionomycin (P/I). Co-localization of Tip60, Ets-2 and NFAT-2 in Jurkat cells ±P/I was investigated by co-immunofluorescence. ChiP analysis was performed to determine Tip60 and Ets-2 binding to the IL-2 promoter. Results: Tip60 overexpression and silencing resulted in the activation and suppression of the IL-2 gene, respectively. Tip60 and Ets-2 interaction and co-localization was observed in CM and P/I conditions whereas Tip60 and NFAT-2 interaction was observed in P/I conditions only. In unstimulated cells, both Tip60 and Ets-2 bound to the ARRE-2 region. In contrast, P/I stimulation resulted in the departure of Tip60 and Ets-2 from ARRE-2 and the binding of Tip60 to the core promoter.Conclusion: Tip60 interacts with Ets-2 in both CM and P/I and with NFAT-2 in P/I conditions. We suggest that Tip60 contributes to the IL-2 transcriptional activation by dissociating Ets-2 from its binding site and permitting NFAT-2 binding to the ARRE-2 element.

P.A5.04.02
Aurora A function in CD8 killing activity

A. Alcaraz-Serna[1], *E. Bustos-Morán[1], N. Blas-Rus[1], S. Iborra[2], F. Sanchez-Madrid[1];*
[1]Servicio de Inmunología, Hospital Universitario La Princesa, Universidad Autónoma de Madrid, Instituto Investigación Sanitaria La Princesa (IIS-IP), Madrid, Spain, [2]Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain.

Aurora A has been studied in cell cycle progression and tumor generation. Recent work has revealed an unexpected function of Aurora A during inflammation and graft versus host disease development. However, the role of Aurora A in CD8+ T cell effector function and its cytotoxic T lymphocytes-mediated antiviral response has not been explored. To study the role of Aurora A in the CD8 lymphocyte cytotoxic function regulation, an in vitro study has been performed by flow cytometry analysis of CD107 surface expression and cytotoxic assays. Additionally, an in vivo analysis was conducted by infecting mice adoptively transferred with OT-I CD8 T cells and challenged with a Vaccinia-OVA infection in the presence of a specific drug inhibitor of Aurora A. Aurora A inhibition leads to an impairment either on the peptide-specific cytotoxicity and on the degranulation ability of CD8+ T cells. This finding was observed both in mice and human. Moreover, Aurora A blockade seems to reduce the transcriptional induction of genes classically related to the effector function of cytotoxic T lymphocytes, such as granzyme B or perforin. Finally, through an in vivo model of Vaccinia infection we have proved that Aurora A is necessary for the CD8+ T cells-mediated antiviral response. We can then conclude that Aurora A activity is a key factor for the cytotoxic T lymphocytes effector function and also for its action against a viral infectious threat.

P.A5.04.03
The role of DNA damage response in modulating functional plasticity of macrophages

A. Bansal, *B. Schumacher;*
CECAD Research Center , University of Cologne, cologne, Germany.

Introduction - The key innate immune players like neutrophils and macrophages generates potent genotoxic species (ROS and NOS) during infection and tissue injury, suggesting interplay and overlap between DNA damage response (DDR) and innate immune response. However, molecular mechanisms linking DDR with innate immune response are still poorly understood. Therefore, we addressed the following questions: 1) Does DDR prime the macrophages, thus predefining immune response to forthcoming stimuli? 2) What are the key molecular players and signaling pathways underlying the interplay of DDR and innate immune response? **Materials and Methods** - Macrophages were primed with low dose of UV light, which causes moderate and uniform DNA damage. Henceforth, UV primed macrophages are referred as "M_{DDR}". To define M_{DDR} activation and polarization, immune phenotyping, seahorse metabolic energetic analysis and *in vitro* functional assays (endotoxin tolerance assay, phagocytosis and gap closure assay) were performed. The phosphoproteomics analysis was carried out to identify key molecular players linking DNA damage and immune response of M_{DDR} cells. **Results** - The M_{DDR} cells express upregulated actin remodeling genes and have alerted metabolic energetics. Our, *in vitro* functional assays demonstrate that M_{DDR} cells are endotoxin tolerant, have increased phagocytosis capacity and secrete cytokines which increases endothelial cells migration. The phophoproteomics data analysis further suggests, the possible role of DDR-induced histone modification and remodeling in functional gain of M_{DDR} macrophages. **Conclusions** Collectively, our findings show that DNA damage response activation is sufficient to prime the macrophage and modulate its immune function.

P.A5.04.04
Recurrent aphthous stomatitis and role of metals in the etiology

J. Bartova[1], *J. Petanová[2], Š. Podzimek[1], M. Janovská[1], M. Libánská[1], H. Tlaskalová[3], Z. Jirásková-Zákostelská[4], L. Izakovičová[5], S. Slezáková[5];*
[1]Institute of Dental Research,GUH and 1st Faculty of Medicine CHarles University, Prague, Czech Republic, [2]General University Hospital and 1st Faculty of Medicine CHarles University, Prague, Czech Republic, [3]Institute of Mikrobiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic, [4]Institute of Microbiology ,Czech Academy of Sciences, Prague, Czech Republic, [5]Clinic of Stomatology and departmment of Pathophysiology,faculty of Medicine MU, Brno, Czech Republic.

Recurrent aphthous stomatitis (RAS) is the most common disease of oral mucosa, affecting 20-25 % of population worldwide. Nevertheless its etiopathplogy remains unexplained. This condition is characterized by multiple recurrent ulcers with circumscribed margins and yellow floors. RAS is considered to be a multifactorial disorder, the immune and genetic predisposition are proposed to play a major role. The aphthaeeruption ccan be initiated by local trauma, some medication, stress and nutritional factors.
This study examined the presence of hypersensitivity to dental and enviromental metals in patients with clinical diagnosis RAS. The group of patients were examined throught medical history, dental examination and by using a modified test of blast transformation for metals - MELISA. 70 patients and 20 healthy controls without history of RAS were enrolled to this study. Metals with the most significant differences between tested 20 metal in lymphocyte reaction were Hg, Zn, Zr and Ni. The study is supported by grant Czech Health Research Council nr. 15-29336-A.

P.A5.04.05
A mobile endocytic network connects clathrin-independent receptor endocytosis to recycling and promotes T cell activation.

E. B. Compeer[1,2], *F. Kraus[2,3], M. Ecker[2], G. Redpath[2], M. Amazier[4], N. Rother[2], P. Nicovich[2], N. Kapoor-Kaushik[2], Q. Deng[5,6], G. Samson[7], Z. Yang[2], J. Lou[2], M. Carnell[2], H. Vartoukian[7], K. Gaus[2], J. Rossy[2,8];*
[1]University of Oxford, Oxford, United Kingdom, [2]University of New South Wales, Sydney, Australia, [3]Monash Biomedicine Discovery Institute, Melbourne, Australia, [4]Garvan Institute of Medical Research, Sydney, Australia, [5]University of New SOuth Wales, Sydney, Australia, [6]Commonwealth Scientific and Industrial Research Organisation, Geelong, Australia, [7]Biotechnologie Institut Thurgau, Thurgau, Switzerland, [8]Biotechnology Institute Thurgau, Thurgau, Switzerland.

Endocytosis of surface receptors and their polarized recycling back to the plasma membrane are central to many cellular processes, such as cell migration, cytokinesis, basolateral polarity of epithelial cells and T cell activation. Little is known about the mechanisms that control the organization of recycling endosomes and how they connect to receptor endocytosis. Here we followed the endocytic journey of the T cell receptor (TCR), from internalization at the plasma membrane to recycling back to the immunological synapse. We showed that TCR triggering leads to its rapid uptake through a clathrin-independent pathway. Immediately after internalization, TCR is incorporated into a mobile and long-lived endocytic network demarked by the membrane-organizing proteins flotillins. Although flotillins are not required for TCR internalization, they are necessary for TCR its recycling to the immunological synapse, TCR its nanoscaled spatial surface distribution, TCR signalling, and efficient primary T cell activation. Collectively, our data supports a model in which a novel endocytic sorting machinery underpinned by flotillins promotes the recycling of internalized TCR complexes to the immunological synapse to coordinate TCR nanoscaled organization that supports efficient T cell activation.

P.A5.04.06
High Arginase-1 levels in neonatal monocytes interfere with bakterizidal functions and production of cytokines

S. Dreschers, *K. Ohl, C. Platen, K. Tenbrock, T. Orlikowsky;*
Children`s University Hospital Aachen, Aachen, Germany.

Introduction: Bacterial infections enhance serum levels of arginine due to protein catabolism. Monocytes and monocytes derived macrophages (MΦ) express the enzyme arginase-1 and iNOS which metabolize arginine. Activity of iNOS and arginase-1 results in opposing effects: either the pro-inflammatory response (NO^- and ROS-production) and enhanced bacterial inactivation or an anti-inflammatory response via production of IL-10 and expansion of Th2-like T-cell subsets. Controlling the arginine metabolism can be predetermining the course of a bacterial infection. **Hypothesis:** High arginase-expression in MΦ of newborn macrophages (CBMΦ) strengthens anti-inflammatory responses compared to PBMΦ.

Material and methods: Polarization of monocyte-derived MΦ from cord blood and from adult peripheral blood (PBMΦ) according to published protocols. FACS-based immunotyping of receptors and metabolic products (ROS- und NO^--concentrations). *In-vitro E.coli* infection assay. Detection of cytokines (ELISA). **Results**: Arginase-1 expression levels in M2a- and M2c-CBMΦ were found three times overexpressed compared to corresponding PBMΦ subtypes ($p < 0.05$). M2- and M1-CBMΦ arginase-1 levels were twice as high as in corresponding PBMΦ subtypes ($p < 0.05$). IL-10-production and -secretion was enhanced in all subtypes of CBMΦ compared to PBMΦ ($p<0.05$). Nonetheless, infection caused a TNF-alpha/IL-10-ratio, biased to the inflammatory state. Infected M1-CBMΦ secreted more IL-6 compared to M1-PBMΦ. In anti-inflammatory subsets (M2-, M2a-, M2c-MΦ), infection-induced NO^--production was comparable. M1-CBMΦ produced less NO^- than M1-PBMΦ. All MΦ subsets featured equal phagocytosis-capacity. Intracellular killing of was unaltered. M2c-CBMΦ ROS production was lowered compared to M2c-PBMΦ. **Conclusion:** Overexpression of Arginase-1 in CBMΦ, is attributed to stronger pro-inflammatory responses. Bactericidal functions were unaffected.

P.A5.04.07
A key role for microRNAs in regulating IL-17 *versus* IFN-g production by gd T cells

*T. Amado[1], N. Schmolka[1], D. Sobral[2], F. Enguita[1], D. Inácio[1], B. Silva-Santos[1], **A. Gomes**[1,3];*
[1]Instituto de Medicina Molecular, Lisbon, Portugal, [2]Instituto Gulbenkian de Ciência, Oeiras, Portugal, [3]Escola Superior de Tecnologia da Saúde de Lisboa, Lisbon, Portugal.

γδ T cells are an important source of the pro-inflammatory cytokines IL-17 and IFN-γ under (patho)physiologic conditions. In the mouse, CD27+ γδ T cells are committed to IFN-γ expression, whereas their CD27- counterparts make IL-17 but are capable of co-expressing both cytokines under inflammatory conditions. We aim to characterize a novel layer of microRNA-mediated regulation of effector γδ T cell differentiation. First, by comparing the microRNA pools of the two CD27-based γδ T cell subsets, we found that miR-146a was selectively enriched in CD27- γδ T cells and restricted their IFN-γ production by targeting Nod1 mRNA. Next, to overcome the caveat of using surface markers, which do not allow isolation of pure populations of IL-17 or IFN-γ producing γδ T cells, we used a double reporter IL-17-GFP: IFNg-YFP mouse strain.
Pure IL-17+ or IFN-γ+ γδ T cell populations were isolated from peripheral lymphoid organs and subjected to next generation sequencing analysis of both microRNA and mRNA repertoires.
This allowed us to identify, for the first time, miRNA and mRNA signatures directly associated with cytokine expression, rather than TCR Vγ usage or maturation markers. Furthermore, differentially expressed miRNAs and mRNAs were bioinformatically integrated into networks that allowed the identification of 6 miRNAs predicted to target key determinants of the IL-17 program; and 3 miRNA candidates for the IFN-γ program of γδ T cells. Ongoing molecular assays provide an unprecedented functional characterization of the impact of microRNAs on the identity and differentiation of effector γδ T cell subsets.

P.A5.04.08
Expression of endogenous Interleukin-36 activity

***A. Jaafar**, M. Nicklin;*
University of Sheffield, Sheffield, United Kingdom.

INTRODUCTION: IL-36 cytokines comprise three agonists, IL-36α, IL-36β, IL-36γ, and antagonist IL-36Ra. IL-36 appears to be an important mediator for inflammation and immunity, particularly in the skin and other epithelia.
Material and methods: We used rhIL-1α, rhN^6-IL-36α and rhTNF at close to saturating concentrations to activate the IL-36 genes. IL-36 mRNAs were detected in both HaCaT (an untransformed human KC line) and HT-29 (a human colorectal carcinoma/epithelial cell line).
RESULTS: RT-PCR and RT-qPCR showed that IL-36β, Il-36γ, IL-36R and IL-36Ra mRNA were expressed in HaCaT in monolayer. Cell de-differentiation and differentiation of HaCaT in monolayer by Ca^{2+} modulation had little effect on the inducibility of IL-36β or IL-36γ. IL-36β, IL-36γ and IL-36R mRNA were also expressed by HT-29 but the standardised levels of IL-36β and IL-36γ were an order of magnitude lower compared with HaCaT. In HaCaT, the inducer cytokines were effective in the order TNF>IL-1>IL-36. In HT29 IL-36 was more effective than IL-1. HaCaT also responded to inducers of IL-8/CXCL8 secretion in the order TNF>IL-1>IL-36. In HT-29, IL-8 secretion was very effectively induced by rhIL-36α.
CONCLUSION: Expression levels of IL-36β and IL-36γ seem to be intrinsically cell line dependent. Maximum expression in HaCaT was an order of magnitude stronger than in HT-29. In HaCaT, dependence of IL-36 expression on even gross changes in cell morphology was weak. In both HaCaT and HT-29, there was a correlation between the proportionate increase in IL-8 secretion and the expression of IL-36 genes, which may reflect relative expression of cell surface receptors

P.A5.04.09
Investigating the post-transcriptional cooperation of Roquin and Regnase-1 in T cell differentiation

***N. Kronbeck**[1], G. Csaba[2], R. Zimmer[2], V. Heissmeyer[1];*
[1]Biomedical Center Munich, Ludwig-Maximilians-Universität, München, Germany, [2]Department of Informatics, Ludwig-Maximilians-Universität, München, Germany.

Gene regulation on the post-transcriptional level exerts essential control over immune responses and is needed to prevent autoimmune diseases.The RNA-binding proteins Roquin-1 and its paralog Roquin-2 were found to be essential for the prevention of autoimmunity and autoinflammation by controlling T cell activation and differentiation. Roquin proteins recognize *cis*-elements in the 3' UTRs of target mRNAs with different "functional" affinity/avidity and induce mRNA decay post-transcriptionally. In T cells, the expression of Roquin proteins themselves is regulated by proteolytic MALT1 cleavage downstream of the T cell receptor in a signal strength-dependent manner. The endonuclease Regnase-1 is regulated in the very same way by MALT1 cleavage. Moreover Roquin proteins and Regnase-1 share a common set of mRNA targets and can cooperate in the regulation of mRNAs. Additionally a conditional deletion of Regnase-1 in mouse T cells leads to a similar autoimmune phenotype as the knockout of Roquin-1/2 in T cells. These findings suggest that increasing TCR signal strength might gradually inactivate Roquin and Regnase1 and thus cause differential target mRNA derepression that specifies cell fate decisions and effector functions of T cells. By comparing conditional knockouts of either Roquin-1/2, Regnase-1 or all three proteins in T cells we want to elucidate how Roquin and Regnase-1 cooperate in the regulation of specific mRNAs and which consequences this cooperation has on cell fate decisions of T helper cells and the development of autoimmunity.

P.A5.04.10
The role of dendritic cells in lactobacilli-mediated dampening of pro-inflammatory immune responses

***G. Lasaviciute**, J. Quin, A. Östlund Farrants, E. Sverremark-Ekström;*
Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden.

During the last years, research has focused on the interplay between the microbiota and the immune system. Lactobacilli, which are frequently found in the human gut, are of particular interest due to their beneficial probiotic properties. Previous results from our group and others suggest that lactobacilli modulate T-cells responses *in vitro*, however, the mechanisms involved are not yet fully understood. Since antigen presenting cells (APCs) like dendritic cells (DCs) are responsible for immune activation, we are interested whether lactobacilli induce epigenetic events in DCs and influence their functional phenotype. Our preliminary results show that 2h stimulation of monocyte-derived DCs with *Lactobacillus (L.) reuteri*-cell free supernatant (CFS) induce maturation of DCs in terms of increased cell surface and gene expression of CD83. We also show that the expression of pro-inflammatory cytokines IL-6 and IL-23 is upregulated in *L. reuteri*-CFS stimulated cells. Further, the results from chromatin immune precipitation (ChiP) experiments show that chromatin accessibility at the promoter region of genes encoding markers important for DC maturation and function clearly differs between *L. reuteri*-CSF and *Staphylococcus aureus*-CFS stimulation. To date, we hypothesize that changes in histone modifications, which can be identified by "activating and silencing marks", and recruitment of different transcription factors at the promoter region of DCs genes might contribute to lactobacilli-mediated immune regulation.

P.A5.04.11
IL-36 is activated by several pathogen-derived proteases and thus functions as a global alarmin of epithelial infection

*J. Ainscough[1], **T. Macleod**[1], M. Stacey[1], M. Wittmann[1,2];*
[1]University of Leeds, Leeds, United Kingdom, [2]Leeds Institute of Rheumatic and Musculoskeletal Medicine, Leeds, United Kingdom.

The interleukin (IL)-1 family of cytokines are fundamental regulators of the innate immune system, pivotal in initiating and orchestrating inflammation. IL-36γ is a recently described IL-1 family member expressed at epithelial barriers that drives immune responses against a range of pathogenic insults. As with other IL-1 family members, IL-36γ is expressed as an inactive precursor that must undergo proteolytic truncation to become biologically active. Initial experiments incubating IL-36γ in pathogenic bacterial and fungal conditioned media illustrated IL-36γ is susceptible to cleavage by pathogen-derived proteases. Importantly, analysis by mass spectrometry showed Aspergillus fumigatus (Af), Trichophyton rubrum (Tr) and Streptococcus pyogenes (Sp) cleave IL-36γ to its potent pro-inflammatory truncation (IL-36γ S18). Subsequent experiments utilising protease-knockout strains and recombinant proteases demonstrated IL-36γ activation is mediated by virulence factors secreted by Af and Sp. Challenging oral epithelial cells with heat fixed Af and Sp increased expression of IL-36γ, whilst addition of live pathogens caused both IL-36γ release and activation. Furthermore, IL-36γ activation was inhibited when experiments were repeated with protease-knockout strains. In summary, these investigations show invasive bacterial and fungal pathogens induce IL-36γ production and release from epithelial cells and secrete proteases which activate the cytokine once it has been released. Given that IL-36γ is activated by such a broad range of pathogen-derived proteases, it is postulated this cytokine functions as a sensor of exogenous protease activity, thus is a broad alarmin of infection. Ultimately, this mechanism may represent an essential mediator of host defence against a variety of important human pathogens.

P.A5.04.12

Nanoscale organisation of the T cell receptor by the chemokine receptor CCR5

A. Martín-Leal[1], *R. Blanco*[1], *J. Casas*[2], *M. T. Rejas*[3], *C. Drechsler*[4], *M. Moreno*[1], *S. Escudero*[1], *W. Schamel*[5], *G. Fabriás*[2], *B. Alarcón*[3], *H. M. van Santen*[3], *S. Mañes*[1];
[1]*Centro Nacional de Biotecnlogía-CSIC, Madrid, Spain,* [2]*Institute of Advanced Chemistry of Catalonia (IQAC-CSIC), Barcelona, Spain,* [3]*Centro Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain,* [4]*Department of Pharmaceutical Technology and Biopharmacy, University of Freiburg, Freiburg, Germany,* [5]*BIOSS Centre for Biological Signalling Studies and Faculty of Biology, University of Freiburg, Freiburg, Germany.*

Chemokines and their receptors are key molecules that coordinate trafficking of lymphocyte subtypes. CCR5, the chemokine receptor for the ligands CCL3, CCL4 and CCL5, is also a costimulatory receptor necessary for maximal activation of $CD4^+$ T cells. This latter activity requires antigen-mediated stimulation of the T cell receptor (TCR) and CCR5 ligand secretion by both the antigen-presenting cell and the T-cell. Although the role of CCR5 in naïve $CD4^+$ T cell activation is well-established, whether it has additional functions in antigen-experienced T cells remains unknown. The TCR is organised in nanoclusters, which are larger in activated and memory than in naïve counterparts. This reorganisation to larger TCR oligomers explains the increased antigen sensitivity of preactivated T-cells, probably because the nanoclusters can be stimulated at lower antigen concentrations than monomeric TCRs. We report that CCR5 activity determines TCR nanocluster size and valency in antigen- experienced T cells. This activity is $CD4^+$ T lymphocyte-specific and independent of CCR5-induced costimulatory signals. CCR5-induced TCR nanoclustering was associated to changes in cell lipid composition. Activated $CCR5^-CD4^+$ T cells had higher ceramide levels than $CCR5^+$ counterparts, coinciding with increased expression of several ceramide synthase isoforms in the $CCR5^-$ cells. In sphingomyelinase-treated live cells and in artificial liposomes, ceramide levels critically determined TCR nanocluster size. Finally, CCR5 deficiency impaired antigen sensitivity of antigen- experienced $CD4^+$ T cells *in vitro*, and reduced T-cell help for immunoglobulin class switching *in vivo*. Our results identify a CCR5 role in TCR nanocluster formation, and suggest CCR5 participation in memory T-cell function.

P.A5.04.13

The Role of heterotrimeric G Proteins in Homing and Migration of Lymph derived T cells

G. E. Patzer[1], *M. Permanyer Bosser*[1], *M. Friedrichsen*[1], *M. Galla*[2], *R. Förster*[1];
[1]*Institute of Immunology, Hannover, Germany,* [2]*Institute of Experimental Hematology, Hannover, Germany.*

The process of lymphocyte homing to the lymph nodes (LNs) via specialized high endothelial venules is well studied while the mechanisms how lymphocytes enter the LN via afferent lymphatic vessels remain largely elusive. Previous studies using intra-lymphatic delivery of cells showed that naïve CD4+ T cells reach the T cell zone (TCZ) of lymph nodes through the medullary sinuses while activated CD4+ T cells can migrate into the TCZ directly through the SCS in a CCR7-dependent manner. However, activated $CCR7^{-/-}$ T cells are still able to enter the LN parenchyma to some extent while pertussis toxin treated T cells are retained in the SCS indicating that other chemokine receptors and/or signaling pathways must be involved in LN homing and intranodal migration. Using the CRISPR-Cas9 technology we now report the generation of activated primary CD4+ T cells deficient for Gαi2, Gαi3 or for both molecules to assess the effect of Gαi-subunit mediated homing and migration of lymph-derived T cells. Preliminary data indicate that single knockout of either Gαi2 or Gαi3 has limited effect on transwell cell migration assays, as well as on the egress of intralymphatically injected T cells from the SCS into the LN parenchyma. The migration distance of Gαi2-deficient T cells from the SCS into the LN parenchyma was reduced while in double knockout T cells the migration into the parenchyma was impaired compared to wild-type cells. However, unlike Pertussis-toxin treated cells, the double knockout T cells were not retained in the SCS.

P.A5.04.14

Dissecting the role of HDAC1 and HDAC2 in the induction of $CD4^+$ T cells with cytotoxic effector function

T. Preglej[1], *P. Hamminger*[1], *L. Andersen*[1], *T. Bulat*[2], *L. Göschl*[3], *R. Tschismarov*[4], *B. Strobl*[2], *C. Bock*[5], *C. Seiser*[6], *W. Ellmeier*[7];
[1]*Division of Immunobiology, Institute of Immunology, CePII, Medical University of Vienna, Vienna, Aus, Vienna, Austria,* [2]*Institute for Animal Breeding and Genetics, University of Veterinary Medicine, Vienna, Austria,* [3]*Division of Rheumatology, Medicine III, Medical University of Vienna, Vienna, Austria,* [4]*Max F. Perutz Laboratories, Vienna Biocenter, Medical University of Vienna, Vienna, Austria,* [5]*Medical Epigenomics Laboratory, CeMM, Vienna, Austria,* [6]*5Max F. Perutz Laboratories, Vienna Biocenter, Medical University of Vienna, Vienna, Austria,* [7]*Division of Immunobiology, Institute of Immunology, CePII, Medical University of Vienna, Vienna, Austria.*

$CD4^+$ T cells with a cytotoxic phenotype ($CD4^+$ CTLs) play important roles in antiviral and antitumor immunity. Recently, we showed that the histone deacetylases HDAC1 and HDAC2 are essential for the maintenance of $CD4^+$ lineage integrity by preventing a Runx3/CBFβ-dependent induction of a cytotoxic program. In the current study, we want to gain further mechanistic insights in the individual role of HDAC1/HDAC2 in the networks controlling $CD4^+/CD8^+$ T cell identity. Since HDAC1/HDAC2-doubly-deficient $CD4^+$ T cells display reduced survival and thus preclude the analysis of these cells in more details, we generated mice with a T cell-specific deletion (*Cd4*-Cre) of the two *Hdac1* alleles and one *Hdac2* allele ($HDAC1^{cKO}HDAC2^{HET}$ mice). $HDAC1^{cKO}HDAC2^{HET}$ $CD4^+$ T cells acquire $CD8^+$ effector functions upon activation, characterized by high production of IFN-γ, and by the increased expression of T-bet, granzyme B and perforin, but not CD8α. Furthermore Runx3 is up-regulated, whereas ThPOK expression is reduced. Activated $HDAC1^{cKO}HDAC2^{HET}$ $CD4^+$ T cells express high levels of CRTAM, a $CD4^+$ CTL marker, and show high cytotoxic activity towards target cells *in vitro*. The induction of the CTL program correlates with a strong increase in phosphorylated STAT1 and inhibition of IFN-γ/STAT1 signaling in $HDAC1^{cKO}HDAC2^{HET}$ $CD4^+$ T cells attenuates up-regulation of $CD8^+$ lineage genes. Finally, in a murine MCMV model, preliminary data indicate that $HDAC1^{cKO}HDAC2^{HET}$ mice display higher levels of virus-specific $CRTAM^+/CD103^+$ $CD4^+$ T cells upon infection. These results demonstrate that HDAC1/HDAC2 play essential roles in the induction of $CD4^+$ T cells with cytotoxic effector function. Supported by FWF (P26193)

P.A5.04.15

Variation of TNF-alphareceptors co-expression on peripheral blood mononuclear cell subset in health

A. Alshevskaya, J. Lopatnikova, J. Zhukova, F. Kireev, ***S. Sennikov****;*
Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation.

The aim of the study was to examine the expression level of type 1 and 2 receptors to TNF-alpha (TNFR1/TNFR2) on main immune subsets of peripheral blood cells.
Methods: The study was conducted on 46 healthy donors (18-77 years). Co-expression and number of TNFR1/2 were calculated for monocytes, B-cells, T-cells, as well as among: cytotoxic T-cells, T-helpers, activated CD8+ and CD4+ cells, memory T-cells and naive T-cells, and T-regulatory cells by flow cytometry analysis (BD FacsVerse, USA).
Results: The highest percentage of double positive cells was in activated cytotoxic T-cells subset (25.8%), slightly lesser - in monocytes (15.2%), cytotoxic T-memory cells (14.3%) and CD5+ B-cells (13.9%),total B-cell pool and activated T-helper (11.5%); for the remaining subpopulations, proportion of double-positive cells did not exceed 10%. For all populations studied, small fraction of cells expressed only TNFR1 (and it was always at least twice lower than percentage of double-positive cells) was observed. The almost complete absence of cells expressing only type 1 receptors (less than 0.5%) was identified for T-regulatory cells, cytotoxic and T-regulatory memory cells and CD5+ B-cells. Conversely, the proportion of cells expressing only type 2 receptors in all studied populations was more than 25% and at least twice the proportion of double-positive cells.
Conclusion: The distribution of TNFR1/2 differed significantly among the main immune subsets, which can lead to different levels and types of cell response to the cytokine. Simultaneous presence of both types of receptors on the cell surface is associated with increase of their expression density.

P.A5.04.16

Immunomodulatory properties of advanced glycation end-products formed from different substrates

M. M. Staniszewska[1,2], *K. Gostomska-Pampuch*[1], *I. Jonik*[2], *A. Bronowicka-Szydełko*[3], *M. Krzystek-Korpacka*[3], *A. Gamian*[1];
[1]*Laboratory of Medical Microbiology, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland,* [2]*Laboratory of Separation and Spectroscopic Method Applications, Centre for Interdisciplinary Research, The John Paul II Catholic University of Lublin, Konstantynow 1J, 20-708 Lublin, Poland, Lublin, Poland,* [3]*Department of Medical Biochemistry, Wroclaw Medical University, Chalubinskiego 10, 50-368 Wroclaw, Poland, Wroclaw, Poland.*

Introduction: Glycation plays a role in pathogenesis of several diseases, including diabetes and cancer. Hyperglycemia and oxidative stress enhance generation and accumulation of Advanced Glycation End-products (AGEs), the heterogeneous adducts with distinct properties. AGEs can initiate inflammatory response, but effect of individual products is not well known. Our goal was to learn about immune response on treatment with AGEs generated from different substrates. **Materials and Methods:** Model AGEs were synthesized on myoglobin using glucose, methylglyoxal, glycolaldehyde, and trans-2-nonenal in dry state or in solution. The cellular effect was tested on PMA-activated THP1 cells treated for 48h with 100 μg/ml of AGEs. Secretion of proinflammatory (IL-6, IL-8, TNF-α, and INF-γ), adaptive immunity (IL-2, IL-4, GC-MSF) and anti-inflammatory factors (IL-10) was assessed in culture medium using Multiplex analysis (Bio-Rad). **Results:** There was distinct response of THP1 cells to AGEs generated under different glycation conditions. GA-AGE was the most potent activator. It enhanced secretion of pro-inflammatory cytokines as well as the adaptive immunity factors. In contrast, the most cited AGEs formed from Glc only slightly enhanced production of some cytokines. Glc-AGE also had no effect on adaptive immunity factors. Cell treatment with MGO-AGE and T2N-AGE had negative effect on secretion of all studied pro-inflammatory and adaptive response factors. Additionally, Glc-, MGO-, T2N-derived AGEs decreased secretion of anti-inflammatory cytokine IL-10. **Conclusions:** Immune response to AGEs is unique for individual products and depends on the substrates or conditions of glycation process. The final effect might reflect the composition of AGEs mixture present in microenvironment.

P.A5.04.17

Blocking the assembly of TLR9 signaling complexes

*A. Javmen, H. Szmacinski, J. R. Lakowicz, **V. Y. Toshchakov**;*
University of Maryland, Baltimore, United States.

TLR9 stimulation activates nuclear factor-κB, leading to production of proinflammatory cytokines. Excessive TLR activation can cause inflammatory disease. This study has examined cell-permeating decoy peptides (CPDP) derived from TLR9 TIR domain. CPDP that included TLR9 TIR AB loop, β-strand B, and N-terminal residues of BB loop, 9R34, inhibited TLR9 signaling most potently. Peptides derived from α-helices C, D, and E (αC, αD, αE) also inhibited TLR9-induced cytokine activation, but were less potent than 9R34. 9R34 did not inhibit TLR2/1, TLR4, or TLR7 signaling. The N-terminal deletion modification of 9R34, 9R34-ΔN, inhibited TLR9 to the same extent as the full length 9R34. Binding of 9R34-ΔN to TIR domains was examined using cell-based FRET/FLIM approach. Cy3-labeled 9R34-ΔN dose-dependently decreased fluorescence lifetime of TLR9 TIR-Cerulean fusion protein. Cy3-9R34-ΔN also bound TIRAP TIR, albeit with a lesser affinity, but not MyD88 TIR; whereas peptide from the opposite TIR surface (αE) bound both adapters and TLR9. Intraperitoneal administration of 9R34-ΔN suppressed the ODN 1668-induced systemic cytokines and lethality in mice. This study identifies an in vivo-potent, TLR9-specific inhibitory peptide that targets both receptor dimerization and adapter recruitment. Location of TIR segments that represent inhibitory peptides suggests that TIR domains of TLRs and TLR adapters interact through structurally homologous surfaces within primary receptor complex, leading to formation of a double-stranded, filamentous structure that can elongate either bidirectionally, from two ends in the presence of TIRAP and MyD88, or unidirectionally, only through the αE end, in TIRAP-deficient cells.
This study was supported by NIH/NIAID grant AI-082299 (VYT).

P.A5.04.18

Elevated Nuclear Lamin A is Permissive for Neutrophil Transendothelial Migration but not for Motility through Dense Collagen I Barriers.

***S. K. Yadav**[1], S. W. Feigelson[1], F. Roncato[1], M. Antman-Passig[2], O. Shefi[2], J. Lammerding[3], R. Alon[1];*
[1]Weizmann Institute of Science, Rehovot, Israel, [2]Faculty of Engineering, Bar Ilan Institute of Nanotechnologies and Advanced Materials, Bar Ilan University, Ramat Gan, Israel, [3]Nancy E. and Peter C. Meinig School of Biomedical Engineering, Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, New York, United States.

Nuclear deformation is a rate limiting step for migratory cells to pass through narrow spaces. Mechanical stability of the nucleus is dictated by lamins, intermediate filament proteins which form a structural network underlying the inner nuclear membrane. Lamin A is a key intermediate filament component of the nuclear lamina which is downregulated during granulopoiesis. When elevated, lamin A restricts nuclear squeezing through rigid confinements. The nuclei of circulating T cells and neutrophils express low levels of lamin A/C. To address the role of nuclear deformability and mechanical stiffness in leukocyte migration through endothelial barriers and collagenous matrices, we used a model system based on DMSO differentiated HL-60 (dHL-60) cells overexpressing CXCR2 which upon in vitro differentiation give rise to neutrophil-like leukocytes which demonstrate robust ability to cross inflamed endothelial cells under shear flow. We then tested how upregulation of lamin A in these dHL-60 cells affects their ability to transit through rigid micron-scale constrictions, endothelial monolayers and collagen I barriers.

P.A5.04.19

Lipopolysaccharide at short-term induces aortic valve thickening and valve hemorrhage in ApoE3*Leiden mice.

***A. van Broekhoven**[1,2,3], P. A. Krijnen[1,3], W. W. Fuijkschot[1,3,4], M. C. Morrison[5], I. P. Zethof[1], W. N. van Wieringen[6,7], Y. M. Smulders[4,3], H. W. Niessen[1,2,3], A. B. Vonk[2,3];*
[1]Department of Pathology, VU University Medical Center, Amsterdam, Netherlands, [2]Department of Cardiothoracic Surgery, VU University Medical Center, Amsterdam, Netherlands, [3]Amsterdam Cardiovascular Sciences, Amsterdam, Netherlands, [4]Department of Internal Medicine, VU University Medical Center, Amsterdam, Netherlands, [5]Department of Metabolic Health Research, The Netherlands Organization for Applied Scientific Research (TNO), Leiden, Netherlands, [6]Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, Netherlands, [7]Department of Mathematics, VU University, Amsterdam, Netherlands.

Background: Recently it was shown that a prolonged treatment of non-atherosclerotic mice for 12 weeks with lipopolysaccharide (LPS), an endotoxin involved in sepsis, induced thickening of the aortic heart valve. As most patients with aortic valve (AV) stenosis do have atherosclerosis, we wanted to study the effect of LPS on AV in an atherosclerotic mouse model on the short term.
Methods: ApoE3*Leiden mice were fed with an atherosclerotic diet and subsequently were injected with either LPS or saline and sacrificed at 2 or 15 days post LPS- or saline-injection. These mice were compared with age-matched non-atherosclerotic C57BL/6 control mice. AVs were assessed for valve size, fibrosis, glycosaminoglycans (GAGs), lipids, calcium deposits, iron deposits and inflammatory cells.
Results: LPS-injection caused a borderline significant increase in average AV thickness at 15 days (32.3 μm) and a significant increase in maximal leaflet thickness (128.4 μm) compared to saline-injected mice (32.3 μm; p=0.076 and 67.8 μm; p=0.013 respectively). LPS-injection however did not significantly affect AV area, fibrosis, GAGs and lipids. Furthermore, no calcium deposits were found. Iron deposits, indicative for valve hemorrhage were observed in one AV from the saline-injected group (9.1 %) and in five AVs from the LPS-injected group (29.4 %). No significant differences in inflammatory cell infiltration were observed upon LPS-injection.
Conclusion: LPS caused a borderline significant AV thickening and significant increased maximal leaflet thickness in atherosclerotic ApoE3*Leiden mice, coinciding with increased valve hemorrhage. These results indicate that systemic infection-related acute inflammation can exacerbate AV stenosis on the short term.

P.A5.05 Initiation of immune responses - Part 5

P.A5.05.01

Mitochondrial hyperpolarization induced by Complex-V restriction maintains naïve CD8⁺ T-cell at check

***M. Berger**, A. Saragovi;*
Hebrew University, Jerusalem, Israel.

Recent thymic emigrants (RTEs) represent an immature T-cell subset characterized by a reduced propensity to proliferate following stimuli. Here we describe the discovery of a metabolic checkpoint modulating T-cells propensity to initiate a response upon priming. We demonstrate that RTEs are phenotypically distinct from mature naïve CD8⁺ T-cells by reduced OXPHOS, increased glycolysis, and substantially elevated mitochondrial membrane polarization. We define mitochondrial complex-V restriction as the mechanism governing these metabolic differences. Following these findings, we show that mitochondrial hyperpolarization, driven by ATP synthase restriction, limits naïve CD8⁺ T-cells propensity to respond to diverse stimuli independent of ATP production. Tracing mitochondrial biogenesis in vivo, we reveal that mitochondrial polarization modulates proliferation capabilities of naïve CD8⁺ T-cells upon priming by regulating the acquisition of mitochondrial biomass. Our study defines mitochondrial hyperpolarization induced by complex-V restriction as a critical checkpoint directly controlling RTEs propensity to proliferate upon stimuli allowing intact T-cell population and diversity.

P.A5.05.02

The role of TCR affinity in the CD4+ T-cell response to mycobacterial infection

***N. Bhattacharyya**[1], L. Daniel[1], C. Counoupas[1], S. A. Stifter[1], P. Bertolino[2], C. G. Feng[1];*
[1]The University of Sydney, Camperdown, Australia, [2]Centenary Institute, Camperdown, Australia.

The differentiation of CD4+ T-cells is essential for the generation of effectors with differential cytokine secretion patterns required for combating distinct organisms. Whilst the influence of innate cytokines on CD4+ T-cell polarisation is well established, the direct role of T-cell receptor (TCR) affinity on the differentiation and effector function of CD4+ T-cells is poorly understood. Studies titrating antigen dose or altering peptide affinities have implicated strong TCR signaling the in the development of both Th1 and Tfh cells. To directly investigate the influence of affinity, we use two transgenic mice lines with high (C24) and intermediate (C7) binding affinity for the same epitope on early antigenic target number 6 (ESAT-6), an immunodominant *M.tuberculosis* antigen. Following infection with recombinant BCG expressing ESAT-6, high affinity CD4+ T-cells underwent earlier activation and expanded to a greater extent. Moreover, high affinity CD4+ T-cells acquired Tfh-like phenotypes earlier than their lower affinity counterparts. Importantly, in contrast to low affinity T cells which traffic rapidly into the red pulp, an area populated with mycobacterium-infected F4/80 macrophages, high affinity CD4+ T-cells preferentially localised to B-cell follicles and sequestered from infected macrophages. Taken together our findings suggest that TCR signal strength can determine the intra-splenic localisation of Ag-specific effector CD4+ T cells. They have important implications on our understanding of the regulation of CD4+ T cell-mediated immunity against persistent intracellular infection in lymphoid tissues.

P.A5.05.03

Transcription factor Ets-2 is involved in the regulation of key lymphotropic factors

***P. Davoulou**, I. Aggeletopoulou, I. Panagoulias, A. Mouzaki;*
Division of Hematology, Department of Internal Medicine, Faculty of Medicine, University of Patras, Patras, Greece.

Introduction: Transcription factor Ets-2 is involved in diverse biological functions and transcriptional regulation. Recent work in our laboratory has shown that in naive T helper cells IL-2 expression is blocked by Ets-2. In this work we studied the role of Ets-2 in the regulation of expression of key lymphotropic factors (NFAT2, NF-κB p65, c-Jun and the kinase CDK10) which play a pivotal role in activation and differentiation of T and B cells. Methods: Jurkat, H938 (T lymphocytic cell lines) and HEK cells (embryonic kidney cell line), were transfected with increasing amounts of an Ets-2 overexpressing vector (pCDNA-ets2) in the presence (P/I) or absence (CM) of mitogens. Ets-2 mRNA overexpression was confirmed by real time PCR. Ets-2 overexpression and lymphotropic factor expression at protein level were assessed by Western blot.

Results: Overexpression of Ets-2 in Jurkat and H938 cells induced NFAT2, NF-κB p65 and c-Jun protein levels under both CM and P/I conditions.
In unstimulated H938 cells, it led to a reduction in CDK10 levels, whereas in stimulated cells Ets-2 over-induced CDK10 levels. In unstimulated HEK cells, increasing amounts of pCDNA-ets2 led to an increase in c-Jun and CDK10 levels; in contrast, in stimulated HEK cells resulted in a reduction in CDK10 and c-Jun levels. Conclusion: Ets-2 is involved in the regulation of expression and synthesis of key lymphotropic factors. Our results set the stage for further studies to elucidate the role of Ets-2 in the regulation of signaling pathways involved in the activation and differentiation of T and B lymphocytes.

P.A5.05.04
Antigen dependent TCR repertoire relation of human cT_{FH} and non-cT_{FH} CD4 T cells

M. Hu[1,2]*, A. Cassotta*[1,2]*, A. Lanzavecchia*[2]*, F. Sallusto*[1,2]*;*
[1]*Microbiology and Immunology, ETH Zurich, Zurich, Switzerland,* [2]*Institute for Research in Biomedicine, Bellinzona, Switzerland.*

Human cT_{FH} (circulating follicular helper T) cells are a subset of CD4 memory T cells identified in peripheral blood that express the follicular homing chemokine receptor CXCR5. Our understandings about the relation between cT_{FH} cells and non-cT_{FH} CD4 memory T cells counterpart, as well as how cT_{FH} relates to *bona fide* lymphoid T_{FH} in regards to function, clonality and lineage still remain incomplete. We seek to study these questions in the context of antigen specific response and by adopting high throughput next generation sequencing to investigate TCR repertoire overlapping.
Firstly, we found out the TCR repertoire relation between cT_{FH} and non-cT_{FH} displayed antigen dependent pattern. C.Albicans, Tetanus toxoid specific cT_{FH} cells were more clonally related with their non-cT_{FH} counterpart, while influenza vaccine specific cells showed a very low level of TCR overlapping between the two. This distinct sharing pattern by flu led us to investigate the possible relation of flu specific cT_{FH} cells with GC T_{FH} cells. *Ex-vivo* stimulation demonstrated the enrichment of Candida, Tetanus specific cells was in the $ICOS^-PD1^-$ cT_{FH} subset, while flu specific cells were remarkably enriched in $PD1^+$ cT_{FH} subset. This was interestingly reflecting the linage relations revealed by TCR sequencing. Future plan is to further discriminate between $ICOS^-PD1^-$ and $PD1^+$ subpopulations of cT_{FH} cells in aspects of their clonal relations with non-cT_{FH} cells, and their phenotypic resemblance with GC T_{FH} cells. This study will reveal the heterogeneity within cT_{FH} cells in terms of antigen specific clonal relations with non-cT_{FH} and its undescribed antigen types dependency.

P.A5.05.05
TCR signaling strength promotes differentiation of human T follicular helper cells

T. Jorritsma[1]*, N. J. Verstegen*[1,2]*, P. A. Unger*[1]*, B. P. Nicolet*[1]*, M. Aalbers*[1]*, A. ten Brinke*[1]*, M. van Ham*[1,2]*;*
[1]*Sanquin Research & Landsteiner Laboratory, Dept of Immunopathology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands,* [2]*Synthetic Systems Biology and Nuclear Organization, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands.*

T follicular helper (Tfh) cells promote antibody diversification and B cell differentiation though their localization in germinal centers, the specific action of costimulatory molecules and the Tfh cytokine IL-21. We previously reported that, in addition to IL-21, human Tfh cells may plastically coexpress the Th1 cytokine IFN-γ and/or the Th2 cytokine IL-4. As IFN-γ directs chemokine expression during B cell differentiation and IL-4 promotes memory B cell formation, it is important to elucidate how cytokine coexpression in Tfh cells is controlled. Here we identified TCR signaling strength as an important regulator of plasticity in the Tfh-like, IL21-producing cell population. IL-21 expression is favored by high TCR signaling in human naive $CD4^+$ T cells. In line with the known preference for Th1 skewing under those conditions, the fraction of IL21-producing cells that coexpress IFN-γ progressively increases with TCR signaling strength. In contrast, coexpression with IL-4 decreases, as also in Tfh cells, IL-4 benefits from low TCR signaling. Similar to the notion that Th1/Th2 polarization is largely mutually exclusive, demonstrated by the inhibitory effect of hallmark cytokines IFN-γ and IL-4 on Th2 and Th1 differentiation respectively, we demonstrate that IL-4 inhibits generation of Tfh-like, IL21-producing cells whereas IL-21 promotes autocrine IL-21 expression, but inhibits expression of the Th2-cytokine IL-4. These data show how formation of Tfh-like, IL21-producing cells and coexpression of other effector cytokines to modulate B cell differentiation is regulated by the magnitude of TCR signaling and availability of IL-4 and IL-21, which may be instrumental to improve vaccine effectiveness.

P.A5.05.06
Antigen-dependence of cell cycle speed during priming shapes CD8 T cell memory

L. Kretschmer[1]*, M. Flossdorf*[1]*, M. Plambeck*[1]*, J. Mir*[1]*, A. Toska*[1]*, Y. Cho*[1]*, I. Treise*[2]*, D. H. Busch*[1,3,4]*, V. R. Buchholz*[1]*;*
[1]*Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München (TUM), Munich, Germany,* [2]*German Mouse Clinic, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany,* [3]*Focus Group "Clinical Cell Processing and Purification", Institute for Advanced Study, TUM, Munich, Germany,* [4]*German Center for Infection Research (DZIF), Munich, Germany.*

Initial antigen encounter triggers T cells to proliferate at speeds close to the physiologic maximum of mammalian cells. T cell memory, on the other hand, is maintained in absence of antigen by exceedingly rare cell divisions. The transition between these fundamentally distinct proliferative programs has been difficult to resolve via population-based analyses. Here we combined an approach for mapping the fate of single CD8 T cells, developed in our laboratory (*1-3*), with the timed depletion of peptide-pulsed Dendritic cells (DCs) *in vivo*. Computational modelling and cell cycle analyses showed that long before reaching peak expansion, slower cycling central memory precursors (CMPs) segregated from rapidly dividing effector subsets. Moreover, timed DC depletion revealed that cycling speed of CMPs was selectively dependent on sustained antigenic stimulation. Accordingly, recall responses were impaired when primary antigen availability was curtailed but not when inflammatory stimuli were reduced. By identifying an antigen-dependent hierarchy of cell cycle speeds among emerging CD8 T cell subsets, we provide crucial mechanistic insights important to guide future vaccination strategies and adoptive T cell therapy.
1. V. R. Buchholz *et al.*, Disparate individual fates compose robust CD8+ T cell immunity. *Science*. 340, 630-635 (2013).
2. P. Graef *et al.*, Serial transfer of single-cell-derived immunocompetence reveals stemness of CD8(+) central memory T cells. *Immunity*. 41, 116-126 (2014).
3. M. Flossdorf *et al.*, CD8(+) T cell diversification by asymmetric cell division. *Nat. Immunol.* 16, 891-893 (2015).

P.A5.05.07
Identification of novel modulators of MR1 trafficking using a gene trap screen in haploid cells

C. Kulicke[1]*, E. De Zan*[2]*, M. Salio*[1]*, P. Klenerman*[3,4]*, S. Nijman*[2]*, V. Cerundolo*[1]*;*
[1]*MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom,* [2]*Ludwig Institute for Cancer Research Ltd. and Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom,* [3]*Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom,* [4]*Translational Gastroenterology Unit, John Radcliffe Hospital, Headington, Oxford, United Kingdom.*

The monomorphic MHC-I-related protein 1 (MR1) presents bacterial metabolites to mucosal-associated invariant T (MAIT) cells, an innate-like subset of T lymphocytes. The known MAIT-activating MR1 ligands are intermediates of riboflavin synthesis, a pathway specific to certain fungi and bacteria and, thus, intrinsically non-self for humans. Here, we use a functional genetic screening technique based on insertional mutagenesis of the near-haploid human cell line HAP1 to discover novel players in MR1 antigen presentation and trafficking. A HAP1 clone overexpressing MR1 was transduced with a gene trap virus to inactivate genes in an unbiased manner. Subsequently, the mutagenised population was treated with the MR1-stabilising ligand Acetyl-6-Formylpterin and stained for MR1 surface expression. The tails of the distribution were FACS sorted to enrich for cells in which positive or negative regulators of MR1 were inactivated. Mapping of the viral insertion sites by Illumina deep sequencing allowed identification of genes statistically overrepresented in either of the two sorted populations which constitute putative modulators of MR1 intracellular trafficking or MR1 stability. The most significant positive regulator of MR1 surface expression identified in our screen was β_2-microglobulin, confirming its indispensable role in the surface translocation of MR1. A number of other putative regulators of MR1 trafficking were taken forward into a CRISPR/Cas9-based validation approach and tested in functional and biochemical assays.
Support: CK - Wellcome Trust studentship; MS, VC - MRC Human Immunology Unit Core Funding; PK - Wellcome Trust (WT109965MA)

P.A5.05.08
Erythrocyte derived interleukin-33 instructs the specification of iron-recycling macrophages

Y. Lu*;*
University of Cambridge, Cambridge, United Kingdom.

Tissue-resident splenic red pulp macrophages (RPM) contribute to red blood cell blood homeostasis by phagocytosing damaged or senescent erythrocytes and releasing heme-associated iron for recycling during erythropoiesis. Heme, a metabolite of erythrocyte degradation, induces the SpiC transcription factor expression in monocyte-derived macrophages, and promotes their differentiation into a precursor pre-RPM phenotype ($CD11b^{hi}$ $F4/80^{lo}$ $SpiC^{lo}$) [Kohyama M, Nature 2009] [Haldar M, Cell 2014]. However, the requirements for differentiation into the mature RPM phenotype ($CD11b^{lo/-}$ $F4/80^{hi}$ $SpiC^{hi}$) remain unknown. Here, we demonstrate that IL-33, co-operates with heme to induce high levels of SpiC in monocyte-derived macrophages, and promotes the generation of mature RPM. Mice deficient for the IL-33 receptor ST2 display a cell-autonomous deficit in monocyte-derived RPM, with a profound phenotypic alteration of the remaining RPM. Consequently, aging ST2-deficient mice have a profound defect in iron-recycling and develop substantial splenic iron deposition. Mechanistically, we show that IL-33 is stored in erythrocytes and is required together with heme, to induce the differentiation of pre-RPM into tissue-resident RPM. Thus, reconstitution of RPM-deficient *Il33*$^{/}$ mice with wild type, but not *Il33*-deficient, erythrocytes substantially restores the generation of RPM. This IL-33-elicited promotion of RPM is dependent on the MyD88 and ERK1/2 pathways downstream of IL-33/IL1RL1 signalling. This work identifies an exquisite co-operation between two microenvironmental cues for the differentiation of a tissue-resident macrophage subset, and it is example of a local cytokine-dependent functional specialisation of a tissue-resident macrophage subset. The work also provides new insights into the role of IL-33/ST2 pathway in iron homeostasis

P.A5.05.10

The potential role of Delta42PD1-TLR4 pathway in augmenting Vdelta2 T cell stimulation of CD4+ T cell response

Y. Mo[1], *A. K. Cheung*[2], *Z. Chen*[1];
[1]*AIDS Institute, Research Center for Infection and Immunity, Department of Microbiology, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong SAR, China,* [2]*Department of Biology, Hong Kong Baptist University, Hong Kong SAR, China.*

Immune system plays a crucial role in different diseases directly or indirectly, for instance, acquired immunodeficiency diseases and cancer progression. PD-1 and its ligands, PD-L1 and PD-L2, negatively regulate the immune response via suppressing T cell functions. In contrast, a novel alternatively spliced isoform of human PD-1, named as Δ42PD1, may serve as a new immune-stimulator due to its characteristics that it does not bind to either PD-L1 or PD-L2 and that it can bind to TLR4. Our recent study found an increased level of Δ42PD1 protein on Vδ2 subset of γδ-T cells in HIV-1 acute patients, which induced intestinal inflammation. To further define the function of Δ42PD1, we show here that activated Vδ2 T cells *in vitro* elevated Δ42PD1 level while co-upregulated MHC class II expression. Since it was reported that Vδ2 T cells can act as an antigen presenting cell, we hypothesize that Δ42PD1 on Vδ2 T cells could play a role in stimulating CD4 T cell responses.
By investigating TLR4 level on $CD4^+$ T cells, our results showed that TLR4 could be upregulated upon pan-activation, with particularly significant high expression on the $CD45RA^+CD45RO^+$ transitional subset of $CD4^+$ T cells. Furthermore, co-culture experiments of virus-induced Δ42PD1$^+$Vd2 T cells and autologous effector cells resulted in the $CD45RO^+CD45RA^+CD4^+$ cells being activated measured by IFN-γ production, which is impaired when blocking antibody against Δ42PD1 was used. Therefore, our study suggests that the Δ42PD1/TLR4 pathway has a novel niche in stimulating the $CD45RA^+CD45RO^+CD4^+$ subset exhibited by Vδ2 T cells.

P.A5.05.11

The environmental exposure is more important than BCG vaccination for the maturation of infant Vγ9Vδ2 T cells

M. Papadopoulou[1,2], *T. Dimova*[2], *W. Hanekom*[3], *E. Nemes*[3], *D. Vermijlen*[1,2];
[1]*Department of Pharmacotherapy and Pharmaceutics, Université Libre de Bruxelles, Brussels, Belgium,* [2]*Institute for Medical Immunology, Université Libre de Bruxelles, Brussels, Belgium,* [3]*South African Tuberculosis Vaccine Initiative, University of Cape Town, Cape Town, South Africa.*

γδ T cells are unconventional lymphocytes sharing attributes of both innate and adaptive immunity. Vγ9Vδ2 T cells, which react towards microbe- and host-derived non-peptidic metabolites (phosphoantigens), are the major γδ T cell population in adult human peripheral blood. Some of their main effector functions, such as IFNγ production, are already programmed before birth. Therefore, we wanted to investigate the effect of an early phosphoantigen encounter on infant Vγ9Vδ2 T cells. For that, blood was collected from 10-week-old infants vaccinated at birth or not with the phosphoantigen-containing BCG vaccine as well as from newborns and adults. We performed flow cytometry assays *ex vivo* or after *in vitro* stimulation of PBMCs and analysis of the γδ TCR repertoire. Our data indicate that there is no significant difference on the phenotype or the effector functions and that there is no outstanding change in the γδ TCR repertoire between the vaccinated and non-vaccinated infants. However, infant Vγ9Vδ2 T cells, independent of their BCG status, showed striking differences compared to their neonatal counterparts such as a high expression of the cytotoxic mediators granzyme B and perforin, which was not observed in other T cell subsets. In conclusion, our data indicate that other environmental encounters of infants are more important than BCG vaccination for the early phenotypic and functional evolution of the Vγ9Vδ2 T cells.

P.A5.05.12

Role of PARP-1 in regulatory Foxp3CD4 T and helper-17 T cell differentiation

F. Novelli, ***C. Pioli****;*
ENEA, Division of Health Protection Technologies, Rome, Italy.

Recent findings highlighted the role of ADP-ribosylating enzymes in inflammation and immune responses, with PARP-1 (poly(ADPribose)polymerase-1) playing a relevant role in leukocyte activation and differentiation. We had found that PARP-1 deficient (PARP-1KO) mice display increased number of regulatory $CD4^+Foxp3^+$ T cells (Tregs) in central as well as peripheral lymphatic organs compared with wild-type (WT) controls. PARP-1KO Tregs were functional as assessed both *in vitro* and *in vivo*. While in a chimera competitive assay PARP-1KO thymocytes generated higher numbers of Tregs compared with WT cells, we wondered whether conversion of naïve CD4 cells to inducible Tregs (iTregs) was also affected. Purified naïve CD4 cells from PARP-1KO mice, stimulated *in vitro* with CD3/CD28 and TGFβ1, expressed Foxp3 mRNA at higher levels and generated a greater number of $Foxp3^+$ iTregs than the WT counterpart. Interestingly, *in vitro* differentiation of purified naive CD4 cells to Th17 cells, as induced by CD3/CD28, TGFβ1 and IL-6, was not affected by PARP-1 deficiency. In peripheral lymphatic organs, conversion to $Foxp3^+$ iTregs occurs upon stimulation by dendritic cells (DCs) in a tolerogenic context. Noteworthy, we found that purified WT naïve CD4 cells, stimulated with CD3 and TGFβ1 in the presence of either WT or PARP-1KO DCs, generated comparable numbers of $Foxp3^+$ iTregs. At variance, WT DCs induced a higher frequency of $IL17^+CD4^+$ cells compared with PARP-1KO DCs. Altogether, these results indicate that PARP-1 plays an important role in the balance between regulatory and Th17 cell differentiation with the involvement of both CD4 T cell intrinsic and DC-mediated effects.

P.A5.05.14

CD1b presents *Borrelia burgdorferi* glycolipid to human T cells

P. Reinink[1,2], *M. Souter*[3], *T. Cheng*[2], *T. van Gorkom*[4,5], *J. Kubler-Kielb*[6], *K. Strle*[7], *K. Kremer*[5], *S. Thijsen*[4], *A. Steere*[7], *D. Pellicci*[3,8], *D. Godfrey*[3,8], *B. Moody*[2], *I. Van Rhijn*[1,2];
[1]*Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands,* [2]*Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital and Harvard Medical School, Boston, United States,* [3]*Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Australia,* [4]*Department of Medical Microbiology and Immunology, Diakonessenhuis Hospital, Utrecht, Netherlands,* [5]*Laboratory for Infectious Diseases and laboratory Surveillance, Centre for Infectious Diseases Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands,* [6]*National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, United States,* [7]*Center for Immunology and Inflammatory Diseases,Massachusetts General Hospital, Boston, United States,* [8]*ARC Centre of Excellence in Advanced Molecular Imaging, University of Melbourne, Melbourne, Australia.*

Lyme disease is caused by the spirochete *Borrelia burgdorferi.* Two ubiquitous lipids of *Borrelia burgdorferi*, BbGL-I and BbGL-II, comprise 35% of the total lipid mass of the bacteria and are specifically synthesized by pathogenic *Borrelia* spp. It is known from previous studies that BbGL-II can bind to CD1d and activate CD1d restricted NKT cells. In this study, we carried out FACS-sorting with CD1b-BBGL-II tetramers, to obtain a T cell line from a Lyme disease patient that recognizes BbGL-II presented by CD1b. The T cell clone binds to CD1b-BbGL-II tetramers but not to mock loaded CD1b tetramers or CD1b loaded with the negative control lipid phosphatidylglycerol. Although there is specific recognition of the CD1b-BbGL-II complex using tetramers, the primary T cells produce comparable levels of IFN-γ in an ELISPOT assay when stimulated with CD1b-expressing antigen presenting cells in the presence or absence of BbGL-II lipid. Whereas the primary T cells preferably bind to CD1b tetramers loaded with BbGL-II, we think that the activation by antigen presenting cells without the addition of BbGL-II is caused by a combination of a low affinity interaction with endogenous lipid-loaded CD1b and a high expression level of CD1b on the antigen presenting cells. We call this phenomenon "antigen-modulated autoreactivity" against the CD1b molecule, where there is an increased reaction to an antigen over a baseline autoreactivity of the T cell towards CD1b. Furthermore, Lyme disease patients were screened for the presence and frequency of CD1b-BbGL-II specific T cells using CD1b tetramers.

P.A5.05.15

The role of the transcription factor Interferon-Regulatory Factor 4 in regulation of Th17 cells

C. Schmidt*, A. Harberts, F. Raczkowski, H. W. Mittrücker;*
Institute of Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

The transcription factor Interferon Regulatory Factor 4 (IRF4) is an essential regulator of $CD4^+$ T cell maturation to different Th cell lineages. As a consequence, IRF4 is essential for effective T cell responses against various pathogens. Here, we aim to characterize the role of IRF4 in Th17 cell differentiation, as well as in the maintenance of the differentiation status and function of these cells. $CD4^+$ T cells from *Irf4*$^{+/+}$, *Irf4*$^{+/-}$ and *Irf4*$^{-/-}$ mice are stimulated under Th17-inducing conditions and cultured for up to three weeks with IL-7 and IL-23. At different time points, the expression of lineage-specific transcription factors and cytokines is determined by intracellular mAb staining and FACS. In addition, $CD4^+$ T cells from *Irf4*$^{fl/-}$ × CreERT2 mice are stimulated and the remaining functional *Irf4*^{fl} allele is subsequently deleted by CreERT2 activation with tamoxifen. First results indicated that induction of Th17 cells strictly depended on the presence of IRF4 during T cell activation, since *Irf4*$^{-/-}$ T cells failed to upregulate RORγt and to produce IL-17A. In contrast, when the functional *Irf4* allele was deleted in $CD4^+$ T cells from *Irf4*$^{fl/-}$ × CreERT2 mice after stimulation, cells retained the capacity to produce IL-17A. So far our results suggest that maintenance of Th17 cells is less dependent on IRF4 than the induction of these cells. Further studies are planned to investigate the role of IRF4 in induction and stability of Th17 cells in vivo in a mouse infection model.

P.A5.05.16
Identification of factors driving induction of liver resident CD8⁺T cells following viral vector vaccination

***A. J. Spencer**, A. Gola, M. Ulaszewska, S. Sebastian, A. V. Hill;*
The Jenner Institute, Oxford, United Kingdom.

$CD8^+$ T cells play a pivotal role in mediating protection from liver-stage malaria, but this protection requires high numbers of $CD8^+$ T cells which are able to locate and kill infected cells during the short time parasites are present in the liver. To improve viral vectored vaccine efficacy, we recently developed a two-step vaccination approach, termed "prime-target", where $CD8^+$ T cells are primed by intramuscular vaccination, followed by an intravenous administration of viral vector, enabling targeting of $CD8^+$ T cells to the liver. This approach leads to high numbers of $CD8^+$ T cells in the liver and much greater levels of efficacy against malaria sporozoite challenge. While both effector and tissue resident (T_{rm}) cells alone can mediate protection, T_{rm} cells are more efficient; we therefore aimed to identify the optimal vaccination regimen for induction of T_{rm} cells.
In this study we tested three different viral vectors, Adenovirus, Modified Vaccinia Ankara and Adeno-Associated Virus, for their abilities to target $CD8^+$ T cells to the liver. In response to all vectors, we observed a correlation between the frequency of antigen specific cells and frequency of T_{rm} cells, however the ratio differed between vectors. Each vector varies in the level and duration of antigen expression and induction of pro-inflammatory signals, suggesting that both of these factors could have an impact on T_{rm} induction. Further experiments are therefore underway to elucidate the impact of antigen level and inflammatory signals on the induction of T_{rm} cells.

P.A5.05.17
Human liver- and skin-derived NK cells exhibit antigen-specific memory responses

*V. Stary, J. Strobl, P. Starlinger, **G. Stary**;*
Medical University of Vienna, Vienna, Austria, Vienna, Austria.

Mounting evidence suggests that NK cells can develop long-lived and highly specific memory to a variety of haptens and viral antigens in mice and in non-human primates. The existence and consequences of antigen-specific NK cell memory still needs to be proven. We isolated NK cells of human livers and blood from individuals vaccinated against hepatitis A and/or B, characterized them phenotypically and functionally in killing assays against antigens the patients had been vaccinated. We evaluated the distribution and function of NK cells in epicutaneous patch test reactions of nickel-sensitized patients, an effector site of adaptive immune responses. In contrast to the peripheral blood, two distinct NK cell populations were found in the liver based on their expression of CD16 and CD49a. $CD49a^+CD16^-$ liver NK cells (54.6% ± 4.2 of total NK cells) performed antigen-specific killing of hepatitis A or B-pulsed autologous B cells matching the patients' vaccination status. Blood-derived and $CD49a^-CD16^+$ liver NK cells did not exert antigen-specific cytotoxicity, but recognized MHC-I^{low} target cells. 57.8 ± 5.1 % of total NK cells in nickel-induced epicutaneous patch tests were found to belong to the $CD49a^+CD16^{low}$ NK cell subset and were capable of specific lysis of nickel-pulsed autologous target cells. These results suggest that antigen-specific memory NK cells in humans are present in the liver and, in case of adaptive immune responses, as effector cells in inflamed skin. The underlying mechanisms for specific recognition of viral antigens and haptens by human memory NK cells might form the basis to target NK cells.

P.A5.05.18
The soluble cytoplasmic tail of CD45 (ct-CD45) in human plasma contributes to keep T cells in a quiescent state

*A. Puck[1], S. Hopf[1], M. Modak[1], O. Majdic[1], P. Cejka[1], S. Blüml[2], C. Arnold-Schrauf[1], J. G. Gerwien[3], K. S. Frederiksen[3], E. Thell[4], J. Leitner[1], P. Steinberger[1], R. Aigner[1], M. Seyerl-Jiresch[1], G. J. Zlabinger[1], **J. Stöckl**[1];*
[1]Institute of Immunology, Medical University of Vienna, Vienna, Austria, [2]Department for Rheumatology, Medical University of Vienna, Vienna, Austria, [3]Novo Nordisk A/S, Biopharmaceuticals Research Unit, Måløv, Denmark, [4]Department for Gynecology, St. Josef Hospital, Vienna, Austria.

The cytoplasmic tail of CD45 (ct-CD45) is proteolytically cleaved and released upon activation of human phagocytes. It acts on T cells as an inhibitory, cytokine-like factor in vitro. Here we show, that ct-CD45 is abundant in human peripheral blood plasma from healthy adults compared with plasma derived from umbilical cord blood and plasma from patients with rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE). Plasma depleted of ct-CD45 enhanced T-cell proliferation, while addition of exogenous ct-CD45 protein inhibited proliferation and reduced cytokine production of human T lymphocytes in response to TCR signaling. Inhibition of T- cell proliferation by ct-CD45 was overcome by co-stimulation via CD28. T-cell activation in the presence of ct-CD45 was associated with an upregulation of the quiescence factors Schlafen family member 12 (*SLFN12*) and Krueppel-like factor 2 (*KLF2*) as well as of the cyclin-dependent kinase (CDK) inhibitor *p27kip1*. In contrast, positive regulators of the cell cycle such as cyclin D2 and D3 as well as *CDK2* and *CDK4* were found to be downregulated in response to ct-CD45. In summary, we demonstrate that ct-CD45 is present in human plasma and sets the threshold of T cell activation.

P.A5.05.19
Resting memory CD4 T cells are generated following prolonged cell division

*J. Sarkander, M. Mursell, Y. Yamasaki, S. Hojyo, **K. Tokoyoda**;*
Deutsches Rheuma-Forschingszentrum Berlin, Berlin, Germany.

CD4 T cell memory is fundamental for long-lasting immunity and effective recall responses following infection or vaccination. We have so far determined that resting memory CD4 T cells specific for systemic antigens preferentially reside in the bone marrow (BM) and that splenic $CD49b^+$T-bet^+ activated CD4 T cells are defined as the precursors of BM memory CD4 T cells. However, it remains elusive how the memory precursors are generated through cell division and interaction with antigen-presenting cells. We here show that the precursors of resting memory CD4 T cells are generated via sustained interaction with dendritic cells and augmented cell division in the late activation phase of a primary immune response. Interestingly, treatment with a cytostatic drug or blockage of CD28/B7 co-stimulatory pathway in the late activation phase abrogates the generation of the memory precursors. Following the sufficient cell division, memory precursors can specifically downregulate CCR7 and upregulate IL-2Rβ, suggesting that loss of CCR7 and gain of IL-2 signaling are required for the migration and survival of the precursors of BM memory CD4 T cells, respectively.

P.A5.06 Initiation of immune responses - Part 6

P.A5.06.01
New insights into mechanisms of sterile inflammation

*N. Freise, A. Burghard, T. Ortkras, N. Daber, T. Vogl, J. Roth, **J. Austermann**;*
Institute of Immunology, Muenster, Germany.

Background: Sepsis is a disease, caused by pathogens, that is still associated with high mortality rates worldwide. After an early strong inflammatory phase, sepsis patients might develop a secondary hypo-reactive state, called endotoxin-tolerance. In this case, invading pathogens cannot be recognized by the immune system and severe secondary infections can arise. However, in 30% of sepsis patients an initial microbial trigger is missing. We recently demonstrated that under sterile conditions endogenous proteins like the alarmins S100A8/A9 are able to induce a hypo-responsiveness of phagocytes, a mechanism we called stress-tolerance.
Objectives: The goal of the present study was to analyze molecular mechanisms underlying stress-induced tolerance in phagocytes and their relevance *in vivo*.
Methods: We investigated the activation of certain signaling pathways in stress-tolerant human or murine phagocytes *in vitro* and *in vivo*. Therefore we performed ImageStream, multiplex, ELISA and western blot analysis and a D-Gal model of septic shock. We also analyzed blood samples of cardiopulmonary bypass patients.
Results: We identified two main signaling pathways to be involved in S100-induced tolerance of phagocytes: the PI3K/AKT/GSK3 and the JAK/STAT pathway. *In vivo* data show a protective effect of a GSK-3 inhibitor on the survival rate of mice during septic shock. Furthermore, master regulator proteins of the JAK/STAT pathway seem to have an important function in phagocytes of cardiopulmonary bypass patients.
Conclusion: The alarmins S100A8/A9 induce stress-tolerance in phagocytes via the PI3K/AKT/GSK3 and the JAK/STAT pathway, relevant for development of a hyporective state in hypoinflammation in cardiopulmonary bypass patients.

P.A5.06.02
Vitamin B complex therapy suppresses neuroinflammation and improves recovery of injured peripheral motor nerve

***B. Bozic Nedeljkovic**[1], S. Dacic[1], P. Nedeljkovic[2], A. Ehmedah[1], B. Draskovic Pavlovic[3], D. Vucevic[3], S. Pekovic[4];*
[1]Faculty of Biology, Belgrade, Serbia, [2]Institute for Orthopedic Surgery "Banjica", Belgrade, Serbia, [3]Military Medical Academy, University of Defense in Belgrade, Belgrade, Serbia, [4]Institute for Biological Research "Sinisa Stankovic", Belgrade, Serbia.

Statement of the Problem: Peripheral nerve injury (PNI) leads to series of cellular and molecular events necessary for axon regeneration and reinnervation of target tissues. Macrophage recruitment that occurs immediately after PNI aids production of cytokines and neurotrophic factors necessary for axon regeneration. Calcium entry via L type of voltage-dependent calcium channels (LVDCCs) is involved in the processes underlying macrophages activation. The aim of this study was to evaluate influence of vitamin B complex therapy on: recovery of motor function after PNI; processes of neuroinflammation that are in part regulated by $Ca_V1.2$ subunit of LVDCCs. Methodology: Adult male AO rats were used. Surgery: Motor branch of femoral nerve was transected and reconstructed by end-to-end anastomosis. Experimental groups: (O) operated, (OT) operated and daily treated with vitamin B complex for 14 days. (S) sham-operated animals, underwent the same procedure but without transection of nerve. Pre- and post-operatively behavior tests were performed. Animals were sacrificed 1, 3, 7, and 14 days post-injury.

Findings: Treatment with vitamin B complex applied immediately after PNI enhanced recovery of walking function, decreased muscle atrophy and improved *musculus quadriceps* activity evaluated by EMG. Additionally, it decreased pro-inflammatory and increased anti-inflammatory cytokines expression and reduced $Ca_v1.2$ expression on activated macrophages. Conclusion & Significance: Vitamin B complex therapy down-regulates expression of $Ca_v1.2$ type of LVDCCs on activated macrophages and suppresses neuroinflammation, thereby contributing to motor function recovery of injured nerve, suggesting possible implementation in therapy of PNI, which remains to be explored.
Acknowledgement: Supported by grants III41014, 175033, MFVMA/10/16-18

P.A5.06.03
TLR antagonist immunosuppressive A151 ODN acts through metabolic reprogramming by suppressing PI3K/AKT/mTOR pathway

O. Bulut[1], *V. Yazar*[1], *G. Kilic*[1], *N. Surucu*[2], *M. Gursel*[2], *I. Gursel*[1];
[1]*Bilkent University, Ankara, Turkey,* [2]*Middle East Technical University, Ankara, Turkey.*

Introduction: A151 ODN, a synthetic oligonucleotide mimetic of telomeric TTAGGG motifs, has great therapeutic potential as an immunosuppressor, not only acting as a TLR9 antagonist, but also downregulating either STAT3 or AIM2. mTOR is a critical downstream element of PI3K/AKT pathway. It is of growing interest how metabolic reprogramming through mTOR regulates immune cell function. Of note, Treg reprogramming is due to targeted mTOR inhibition leading a shift from glycolysis to FA oxidation. This study aims to reveal A151 ODN's action on metabolic reprogramming through mTOR pathway.
Methods: Mouse splenocytes were stimulated with i) mu-TLR9 ligand, ii) A151 or iii) their combinations. Also, fresh PBMC were stimulated similarly with i) hu-TLR9 ligand ii) A151 and iii) their combinations for 2-8h. Status of several mTOR pathway elements were assessed by qRT-PCR, WB and flow cytometric assays.
Results: Data revealed that A151 downregulated mTOR and several downstream targets such as Eif4e and Rps6ka1, both in the absence and presence of CpG ODN. A151 also downregulated PI3Kcs delta and AKT activator Pdpk1. A151 treatment reduced phosphorylation of mTOR and several targets (4ebp1, Foxo1 and Foxo3) while increasing AMPK phosphorylation, a repressor of mTOR. In PBMCs, A151 downregulated expression of mTOR and Glut1 which regulates glycolytic capacity.
Conclusion: These data suggests that inhibitory role of A151 ODN might be dependent on mTOR pathway. We propose that Treg promoting capacity of A151 ODN could be through metabolic programming via mTOR suppression.

P.A5.06.04
Implication of annexin A1 in Lupus Nephritis

F. Dhaffouli[1], *H. Hachicha*[2], *H. Mnif*[3], *N. Elloumi*[1], *S. Feki*[2], *R. Fakhfakh*[1], *T. Boudawara*[3], *k. Kammoun*[4], *H. Masmoudi*[2];
[1]*Autoimmunity and Immunogenetics unit UR12SP14, Habib Bourguiba University Hospital, Sfax, Tunisia, Sfax, Tunisia, Tunisia,* [2]*Immunology Department, Habib Bourguiba University Hospital, Sfax, Tunisia, Sfax, Tunisia, Tunisia,* [3]*Anatomopathology Department, Habib Bourguiba Hospital, Sfax, Tunisia, Sfax, Tunisia, Tunisia,* [4]*Nephrology Department, University Hospital; Renal pathology unit 12ES14, faculty of medecine of Sfax, Tunisia, Sfax, Tunisia, Tunisia.*

Systemic lupus erythematosus (SLE) is an autoimmune disorder that can affect skin, joints, kidney, and central nervous system. Lupus nephritis (LN) is one of the most serious manifestations of SLE.
Annexin A1, a protein coded by the ANXA1gene, is implicated in apoptosis. Previous studies showed high expression of AnxA1 in different nephropathy diseases.
Our aim was to investigate the genetic polymorphism of AnxA1 in SLE and to determine the expression of annexin A1 in renal biopsies of SLE patients.
Our study included 94 lupus patients and 120 matched controls. Polymorphism of AnxA1 [rs2811226] was genotyped using a PCR-RFLP technique.
AnxA1 expression in kidney was carried out on 28 renal biopsy of LN patients and 8 renal tissues obtained from the normal part of nephrectomized kidney and cadaver kidney (autopsy) from subjects without inflammatory renal disease, used as controls. Biopsies were stained by immunohistochimestry.
In SLE patients, the G allele of rs2811226 SNP was significantly increased ($p<0.05$). The expression of this allele was associated with LN ($p=0.044$).
AnxA1 expression hasn't showed any significant difference in neither glomeruli nor tubules between patients and controls. However, the intensity of AnxA1 expression in glomeruli was significantly lower in class II compared with proliferative classes: class III ($p=0.050$) and class III+V, IV + V ($p=0.029$). These findings suggest that AnxA1 could play a role in the persistence and progression of glomerular disorders.
Our results show that ANXA1 could serve as a severity marker in LN and help clinician for the monitoring of lupus patients.

P.A5.06.06
Aberrant VISTA expression on CD45RA$^+$CD25^{dim} Th-cells in Giant Cell Arteritis

R. Hid Cadena, *R. D. Reitsema, W. H. Abdulahad, M. Huitema, A. M. Boots, P. Heeringa, E. Brouwer;*
University Medical Center Groningen, Groningen, Netherlands.

Background: The replenishment of naïve T-cells is severly restricted by thymic involution with ageing. In the past, our group identified CD45RA$^+$CD25^{dim} Th-cells as a subset of post-thymically expanded naïve Th-cells in healthy aged individuals. Immune homeostasis of naïve Th-cells is important in defective immune responses in age-related immune-disorders such as Giant Cell Arteritis (GCA). Recently, a loss of immunoinhibitory checkpoints has been implicated in GCA. The possible contribution of Immune Checkpoint (IC) pathways to the dysregulation of Th-cells, especially in CD45RA$^+$CD25^{dim} Th-cells in GCA has not yet been studied.
Objectives: We aimed to investigate the expression of different IC molecules on circulating CD45RA+CD25^{dim} Th-cells of GCA-patients and compare it with matched healthy controls (HCs).
Methods: In a cross-sectional study, fresh blood samples were obtained from 33 GCA-patients with/without immunosuppressive treatment (glucocorticoids) and 12 sex/age-matched HCs. The frequency of the expression of different IC including CD28, Cytotoxic T-Lymphocyte-associated antigen-4 (CTLA-4), Programmed death- 1 (PD-1), and V-domain Ig suppressor of T-cell activation (VISTA) were determined on CD45RA+CD25^{dim}CD4+Th-cells of GCA-patients and HCs by flow cytometry.
Results: Proportion of circulating CD45RA+CD25^{dim}Th-cells in GCA-patients was not different when compared to HCs. The frequency of CD28, CTLA-4 and PD-1 expression on CD45RA+CD25^{dim}Th-cells also remained unchanged. Interestingly, proportion of VISTA expression on these cells was significantly decreased in GCA-patients.
Conclusions: In GCA-patients, lower frequencies of VISTA+CD45RA+CD25^{dim}Th-cells were noted. Decreased VISTA expression in GCA-patients could play a role in the regulation of Th-cell activation or inhibition. The functional consequences of IC modulation within particular subsets requires further investigation.

P.A5.06.07
Propranolol influences EAE development by impairing antigen presenting cell migration into the draining lymph nodes

I. Vujinovic[1], *I. Pilipovic*[1], *R. Petrovic*[1], *Z. Stojic-Vukanic*[2], *N. Arsenovic-Ranin*[2], ***G. M. Leposavic***[2];
[1]*Institute of Virology, Vaccines and Sera "Torlak", Belgrade, Serbia,* [2]*University of Belgrade-Faculty of Pharmacy, Belgrade, Serbia.*

Introduction: Catecholamines are implicated in development of multiple sclerosis and EAE in Dark Agouti (DA) rats. To enlighten their β-adrenoceptor-mediated immunomodulatory action, DA rats of both sexes immunized for EAE were subjected to seven-day-long treatment with propranolol (β-adrenoceptor blocker) starting at the day of immunization. Methods: The migration of antigen presenting cells (APCs) into the draining lymph nodes (dLNs) was examined using CFSE-based assay. Frequency of activated CD4+ T cells, their proliferation and frequency of IL-17-producing CD4+ T (Th17) cells in dLNs, and their infiltration into spinal cord, were analyzed using flow cytometry. Propranolol effects on CD4+ cell proliferation and Th17 cell polarization, and IL-2 and Th17 polarizing cytokine and chemokine expression in dLNs/ dLN cell cultures were examined using flow cytometry and ELISA/qRT-PCR. Results: Irrespective of sex, propranolol reduced the incidence and postponed clinical EAE onset by impairing migration of antigen-carrying APCs into the dLNs (due to diminished dLN CCL19/21 expression). Consequently, propranolol diminished CD4+ T-cell activation/proliferation, and Th17 cell number in dLNs and spinal cord. To corroborate these findings, propranolol exerted stimulatory effects on CD4+ cell proliferation (through stimulation of IL-2 secretion) and Th17 cell differentiation (reflecting enhanced Th17 polarizing cytokine production) in dLN cell cultures. Conclusions: Irrespective of sex, the stimulatory effects of propranolol on dLN CD4+ T lymphocyte proliferation and activated/matured APC Th17 polarizing capacity were insufficient to overcome its inhibitory influence on APC migration, so propranolol impaired Th17 generation in dLNs of EAE rats, and postponed EAE development. (Grant 175050, MESTD, The Republic of Serbia).

P.A5.06.08
Fcμ receptor as a new costimulatory molecule for T cells

A. Meryk[1], *L. Pangrazzi*[1], *M. Hagen*[1], *F. Hatzmann*[1], *B. Jenewein*[1], *B. Jakic*[2], *N. Hermann-Kleiter*[2], *G. Baier*[2], *J. Jylhävä*[3], *M. Hurme*[4], *K. Trieb*[5], *B. Grubeck-Loebenstein*[1];
[1]*Institute for Biomedical Aging Research, Innsbruck, Austria,* [2]*Division of Translational Cell Genetics, Innsbruck, Austria,* [3]*Department of Medical Epidemiology and Biostatistics, Stockholm, Sweden,* [4]*Faculty of Medicine and Life Sciences, Tampere, Finland,* [5]*Department of Orthopedic Surgery, Wels, Austria.*

The Fc receptor for IgM (FcμR) regulates cellular activation of various cell types. FcμR deficient mice display dysregulated function of neutrophils, dendritic cells, and defects in B cell homeostasis. In contrast, the function of FcμR on human T cells is still unclear. We found that FcμR is mostly stored inside the cell and that surface expression is tightly regulated. Decreased surface expression was found on T cells from elderly individuals, which was associated with alterations in the methylation pattern of the *FCMR* gene. Binding and internalization of IgM stimulates transport of FcμR to the cell surface to ensure sustained IgM uptake. Concurrently, IgM accumulates within the cell and the surface expression of other receptors increases, among them, the TCR and costimulatory molecules. This leads to enhanced TCR signaling, proliferation and cytokine release, in response to low, but not high doses of antigen. Our findings indicate that FcμR is an important regulator of T cell function and reveals a new mode of interaction between B and T cells.

P.A5.06.09

Human bone marrow adipocytes display distinct immune regulatory properties

C. Miggitsch[1], *B. Jenewein*[1], *A. Meryk*[1], *K. Trieb*[2], *W. Zwerschke*[1], *B. Grubeck-Loebenstein*[1];
[1]*Institute for Biomedical Aging Research, Innsbruck, Austria,* [2]*Department of Orthopedic Surgery, Klinikum Wels, Wels, Austria.*

The bone marrow (BM) is a primary lymphoid organ of the human immune system where T and B cell precursors are generated and antigen-experienced adaptive cells are maintained. The BM has proven to be a major reservoir of resting memory T cells and long-lived plasma cells, capable of providing protection against recurrent infections. The survival and maintenance of these cells is mediated by cytokine and chemokine producing stromal cells and myeloid cell types, forming specific areas known as BM niches. However, no information is yet available on the production of memory T cell survival factors by BM fat tissue and the interaction with adaptive immune cells in the BM. Using microarrays, we show that bone marrow fat significantly differs from subcutaneous fat regarding specific gene expression profiles including inflammatory responses. Reduced expression levels of the adipocyte-specific genes may suggest that the BM is an immune regulatory organ. Higher expression of the effector/memory T cell survival factors IL-7 and IL-15 were found in BM compared to subcutaneous adipocytes. The expression of the pro-inflammatory molecules TNFα and IL-6, which contribute to the low-grade inflammatory background known as “inflamm-aging” observed in elderly persons, was also higher in BM fat. With our data, we can show that the unique phenotype of BM adipocytes expressing pro-inflammatory cytokines may have a negative effect on long-lived plasma cells while maintaining effector/memory T cells.

P.A5.06.10

The role of thioredoxin interacting protein (TXNIP) in T cell activation

S. Nagel[1], *S. Ziola*[1], *P. Krammer*[1], *K. Gülow*[1,2];
[1]*German Cancer Research Center (DKFZ), Heidelberg, Germany,* [2]*University Hospital Regensburg (UKR), Regensburg, Germany.*

T cells undergo rapid proliferation and differentiation upon stimulation of the T cell receptor (TCR). We have shown that regulation of TCR-induced oxidative signaling is crucial for control of a T cell immune response. TXNIP is a negative regulator of the oxidative defense controlling the intracellular redox equilibrium. Thus, TXNIP is a promising candidate for regulating T cell signaling. We demonstrate that TXNIP is downregulated independently of ROS upon TCR activation. To examine the molecular mechanism and the resulting effects of TXNIP downregulation upon TCR triggering a CRISPR-Cas9 knockout of TXNIP (TXNIP KO) in Jurkat T cells was generated. By means of TXNIP KO clones the role of TXNIP in T cell activation was addressed. Thereby, we have shown that TXNIP deficiency has no impact on activation-induced oxidative signaling. Nonetheless, we could determine that TXNIP KO T cells show enhanced CD95 death ligand (CD95L/FASL/APO-1-L) expression as well as activation-induced cell death (AICD) upon TCR triggering. Therefore, TXNIP seems to have an impact on activation-induced transcription factors and thus, gene target expression. Hence, a better understanding of the role of TXNIP in T cell activation will give new insights into physiological processes like regulation of a T cell immune response. This knowledge is helpful in modulation of T cell responses to prevent autoimmunity or to activate T cells in a tumor setting. The work was supported by the European Union (FP-7 Health, SyStemAge) and the Wilhelm-Sander-Stiftung (2012.077.2).

P.A5.06.12

Identification of human self-reactive iNKT cells

J. PERROTEAU[1], *L. Hesnard*[1], *M. Devilder*[1], *B. Navet*[1], *L. Gapin*[2], *E. Scotet*[1], *L. Gautreau-Rolland*[1], *X. Saulquin*[1];
[1]*Centre de Recherche en Cancerologie et Immunologie Nantes-Angers, Nantes, France,* [2]*National Jewish Health - University of Colorado, Denver, United States.*

Invariant Natural Killer T (iNKT) lymphocytes express both NK receptors and a semi-invariant αβ TCR restricted by the CD1d molecule presenting glycolipids. Among them, αGalactosylCeramid (αGC) is a potent ligand of all iNKT cells. In some contexts, iNKT cells are also able to detect endogenous glycolipids, which highlights their self-reactivity. However, the mechanisms underlying this autoreactivity are still poorly understood.
By using a tetramer-associated magnetic approach, we generated several iNKT cell lines from the peripheral blood of healthy donors. These cell lines reacted similarly against hCD1d+ target cell lines after loading with aGC, but in a different manner against unloaded hCD1d+ target cells, expressing endogenous glycolipids, both? in terms of cytotoxicity and cytokines production. Moreover we demonstrated that the auroreactivity is dependent on TCR-CD1d signaling. The analysis at the clonal level (n=12) of an autoreactive cell line also revealed an important heterogeneity between clones in terms of self-reactivity. We found identical alpha and beta chain TCR sequences in all the clones obtained, suggesting that expression of particular TCR sequence is not sufficient to induce self-reactivity. Comparative RNAseq analysis revealed a direct correlation between the tyrosine kinase SYC expression-level and autoreactivity, while an inverse correlation was observed with the expression of the phosphatase DUSP2. As SYC and DUSP2 are respectively implicated in TCR signal transduction and modulation of Th17 cells development (Muro et al., 2018; Lu et al., 2015), our results suggest that the balance of expression of these 2 proteins could modulate the intrinsic autoreactive potential of human iNKT cells.

P.A5.06.13

Neutrophils driving unconventional T cells are essential for resistance to sarcomas

A. Ponzetta[1], *M. Barbagallo*[1], *R. Carriero*[1], *M. Molgora*[1], *C. Perucchini*[1], *S. Carnevale*[1], *E. Magrini*[1], *F. Gianni*[1], *P. Kunderfranco*[1], *N. Polentarutti*[1], *F. Pasqualini*[1], *S. Di Marco*[1], *D. Supino*[1], *S. Pilotti*[2], *E. Bonavita*[1], *M. Galdiero*[1], *C. Garlanda*[3], *A. Mantovani*[3], *S. Jaillon*[3];
[1]*Humanitas Clinical and Research Center, Rozzano, Italy,* [2]*Fondazione IRCCS Istituto Nazionale Tumori, Milano, Italy,* [3]*Humanitas University, Pieve Emanuele, Italy.*

Neutrophils represent a fundamental mechanism of antimicrobial resistance and inflammation. Moreover, neutrophils have emerged as important players in the activation, orchestration and regulation of adaptive immune responses. Neutrophils are a component of the tumor microenvironment (TME) and have been shown to promote progression. On the other hand, unleashed neutrophilic effectors have also been reported to mediate anti-cancer resistance. Antibody-mediated depletion used to investigate the role of neutrophils in tumor progression suffers from limitations, including duration, specificity and perturbation of the system. We therefore used a genetic approach to investigate the role of neutrophils in primary 3-methylcholanthrene (3-MCA)-induced sarcomagenesis. Neutrophils were found to play an essential role in resistance against primary carcinogenesis by driving an interferon-γ dependent type 1 immune response. Neutrophils selectively caused type 1 polarization of $CD4^-$ $CD8^-$ unconventional T cells (UTCs) in the TME. In human undifferentiated pleomorphic sarcomas (UPS), unlike other sarcomas, granulocyte-colony stimulating factor receptor *(CSF3R)* expression and a neutrophil signature were associated with better outcome and with a type 1 immune response. Thus, neutrophils, by driving a type 1 immune response and polarization of UTCs, mediate resistance against murine and human sarcomas.

P.A5.06.14

Investigating the molecular basis of Roquin-mediated control of T cell fate decisions

H. Schmidt[1], *V. Heissmeyer*[1,2];
[1]*Ludwig-Maximilians-Universität (LMU), Biomedical Center (BMC), Institute of Immunology, Planegg-Martinsried, Germany,* [2]*Helmholtz Zentrum, Munich, Germany.*

Post-transcriptional gene regulation by RNA-binding proteins (RBPs) controls T cell fate decisions. The RBPs Roquin-1 and -2, encoded by the genes *Rc3h1* and *Rc3h2,* serve redundant functions in T cells. They bind via their ROQ domain to 3′-untranslated regions (3′-UTRs) of mRNAs and control mRNA stability and expression. Interestingly, *sanroque* mutant mice harboring a single ROQ domain point mutation or mice lacking Roquin-1/2 protein expression revealed accumulations of Tfh and Th1 or Tfh and Th17 effector T cells, respectively. Since the Roquin-1^{san} protein is a functional hypomorph of Roquin-1, we hypothesize that the molecular regulation of Th1 versus Th17 differentiation is determined by a graded loss-of-function of Roquin. Therefore, we investigate the effects of (i) graded Roquin reduction by increasing deletion of *Rc3h1* and *Rc3h2* alleles, by (ii) introducing ROQ domain mutations that attenuate affinities to mRNA targets and (iii) by pharmacologically modulating MALT1-induced cleavage of Roquin or stimulating T cells with increased TCR signal strength. Our preliminary data indeed indicate that graded loss of Roquin correlates with increased effector-memory phenotypes of CD4 and CD8 T cells and *ex vivo* analyses suggest graded target upregulation including ICOS, Ox40 and CTLA-4 transcripts. Furthermore, minimal Roquin amounts were still effective to suppress Th17 differentiation as measured by IL-17A and RORyt expression. In future studies, we will seek to uncover how graded T cell stimulation regulates differential Roquin activities that mediate T lymphocyte fate-specifiying mRNA repression.

P.A5.06.15

Noradrenaline synthesized locally in draining lymph node cells modulates CD4+ T-cell development in rat EAE model: a role for α_1-adrenoceptor

I. Pilipovic[1], *I. Vujnovic*[1], *R. Petrovic*[1], *D. Kosec*[1], ***Z. Stojić-Vukanić***[2], *G. Leposavic*[2];
[1]*Institute of Virology, Vaccines and Sera ”Torlak”, Belgrade, Serbia,* [2]*University of Belgrade - Faculty of Pharmacy, Belgrade, Serbia.*

Introduction: It has been suggested that: i) noradrenaline synthesis in ,,adrenergic“ immune cells changes during development of EAE and multiple sclerosis and ii) noradrenaline influences EAE development through α_1-adrenoceptor. To elucidate mechanisms standing behind this phenomenon, α_1-adrenoceptor-mediated influence of draining lymph node (dLN) cell-derived noradrenaline on CD4+ T-cell response in dLNs from Dark Agouti rats of both sexes immunized for EAE was examined. Methods: Cells recovered from dLNs on 7^{th} day post-immunization were examined for noradrenaline synthesis/content and α_{1B}-adrenoceptor expression using HPLC and/or flow cytometry. Additionally, effects of prazosin (α_1-AR blocker) on CD4+ T-cell proliferation, the frequency of IL-17+ CD4+ T-cells and regulatory (Foxp3+CD25+) CD4+ T-cells (Tregs), activational/maturational molecule expression on antigen presenting cells (APCs) and their cytokine profile in dLN cell culture were examined using flow cytometry and/or qRT-PCR/ELISA. Results: Irrespective of sex, conventional CD4+ T-cells and Tregs, and APCs from rat dLNs synthesized noradrenaline, while only Tregs and APCs expressed α_{1B}-adrenoceptor.
In myelin basic protein-stimulated dLN cell cultures from rats of both sexes prazosin increased Treg frequency and Foxp3 expression, but diminished co-stimulatory CD80 and CD86 molecule expression on APCs, thereby reducing CD4+ cell proliferation.

Additionally, prazosin diminished expression of Th17 polarizing cytokines (IL-1β and IL-23) in dLN cell cultures, and reduced the frequency of all IL-17+ CD4+ T-cells, and those coexpressing GM-CSF. Conclusions: The study indicates that in rats of both sexes immunized for EAE, dLN cell-derived noradrenaline through α_1-adrenoceptor influence generation of (auto)immune IL-17+ CD4+ T-cell response and thereby EAE development. (Grant 175050, MESTD, Republic Serbia).

P.A5.06.16

Increased expression of checkpoint inhibitors on CD4⁺ T-cells during CPS immunisation is associated with slower acquisition of immunity

X. Yap[1], *J. Walk*[1], *I. J. Reuling*[1], *W. Gramans*[1], *G. van Gemert*[1], *R. Siebelink-Stoter*[1], *M. van de Vegte-Bolmer*[1], *K. Teelen*[1], *E. M. Bijker*[1,2], *A. Scholzen*[1,3], *R. W. Sauerwein*[1];
[1]Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, Netherlands, [2]Department of Pediatrics, Radboud University Medical Center, Nijmegen, Netherlands, [3]Innatoss Laboratories B.V., Oss, Netherlands.

Malaria poses a significant burden to global health, yet much remains unknown about the development of immunity to the malaria parasite *Plasmodium falciparum*. Chloroquine chemoprophylaxis with *P. falciparum* sporozoites (CPS) immunisation induces highly effective sterile protection in naïve volunteers. However, some volunteers are protected after the first immunisation (fast responders), whereas others require two or more immunisations to be fully protected (slow responders). Checkpoint inhibitors are regulatory molecules which inhibit immune responses, including acquisition of adaptive immunity. Blocking checkpoint inhibitors drastically improves survival in murine malaria studies. However, the importance of checkpoint inhibitors in malaria vaccination has not yet been established.
Expression of checkpoint inhibitors CTLA4, TIM3, and PD-1 was measured by flow cytometry on CD4⁺, CD8⁺, and γδ T-cells, and NK cells from 32 immunised volunteers in two CPS immunisation trials (NCT02080026, NCT02098590). Expression of TIM3 on CD4⁺ and CD8⁺ T-cells (p=0.0167, p=0.0152) and CTLA4 on CD8⁺ T-cells (p=0.0255) differed significantly between fast and slow responders. Furthermore, when expression of all inhibitory markers was summed into a cumulative inhibitory Z-score, CD4⁺ T-cells from fast responders had a significantly lower inhibitory score (p=0.0234) compared to slow responders.
This study demonstrates for the first time that fast responders to malaria vaccination have lower checkpoint inhibitor expression than slow responders. Further studies will examine whether individuals vary in their capacity to express checkpoint inhibitors and produce immunosuppressive cytokines after restimulation with *P. falciparum*-infected erythrocytes. These findings provide insight into how individual immunosuppressive profiles prior to vaccination can affect malaria vaccination efficacy.

P.A5.06.17

CHARACTERIZATION OF HUMAN MONOCLONAL ANTIBODIES AGAINST DENGUE VIRUS NS1 PROTEIN

K. Siriporn, *Pannamthip Pitaksajjakul, Pongrama Ramasoota, Khwanchit Boonha, Wilarat Poungmanee;
Tropical Medicine, Bangkok, Thailand.*

Background: Dengue hemorrhagic fever caused by dengue viruses is a public health problem in tropical and subtropical regions. Today, dengue is considered one of the most important arthropod-borne viral diseases in humans in terms of morbidity and mortality. The dengue virus (DENV) non-structural 1 (NS1) protein plays a critical role in viral RNA replication and has a central position in DENV pathogenesis. During the last three decades, the DENV NS1 protein has also been intensively investigated as a potential target for vaccines and immunotherapy. However, anti-NS1 antibody was recently interested as one factor of severe dengue infection due to their cross-reactivity with human molecules such as endothelial cell, integrin and plasminogen, causing some severe symptoms like vascular leakage. Project description: To identify anti-NS1 human monoclonal antibodies (HuMAbs), in this study, HuMAbs were generated by hybridoma technology by fusing of human PBMCs with human fusion partner cell (SPYMEG). HuMAbs specific for NS1 protein of dengue virus were screened and confirmed by western blot analysis. Target epitope of anti-NS1 was also determined by random peptide phage display. Genetic information of those anti-NS1 HuMAbs was elucidated. Conclusion: This is the first study described the generation and characterization of full IgG human monoclonal antibody specific to NS1 protein of Dengue virus. These characterizations could be used for a study of DENV pathogenesis and dengue vaccine constituents in the future.

P.A5.06.18

Human oral epithelial cells inhibit Th1 cell responses in a cell contact-dependent manner

J. L. Sanchez-Trincado Lopez, *M. Gomez-Perosanz, M. Molero-Abraham, E. M. Lafuente, P. A. Reche;
Department of Immunology, School of Medicine, Madrid, Spain.*

The oral mucosa is a site of intense immunological activity, where tolerogenic and defensive responses are articulated. The underlying mechanisms resulting in active immunity or tolerance are poorly understood but it is evident that oral epithelial cells (OECs) of the mucosa ought to play an important role. Here, we have characterized the ability of human oral squamous cell carcinoma cell lines and primary oral epithelial cells to modulate immune responses. OECs constitutively express CD40 and respond to inflammatory stimulation by increasing MHC II expression without CD80 and CD86 costimulation. Co-culture of OECs with dendritic cells (DCs) drastically reduced IL-12 released by DCs after exposure to bacteria and induced a tolerogenic phenotype characterized by reduced MHC II, CD80 and CD86 expression and increased IL10 production in the presence of primary OECs. OEC-conditioned DCs were unable to promote Th1 differentiation as determined by a lack of IFNγ production in allogenic activated CD4⁺ T cells. Moreover, OECs were able to abrogate CD25 and CD69 expression, T cell proliferation and the release of IFNγ and TNFα when co-cultured with anti-CD3+anti-CD28 stimulated CD4⁺ T cells. The inhibition on T cell activation was TGF-β independent but cell-contact dependent. Our data indicate that the oral epithelium promotes an anti-inflammatory state by conditioning DCs maturation and by dampening Th1 cell responses through direct epithelial-T cell contact.

P.A5.06.19

Phenotype of monocyte-derived dendritic cells in response to halophilic archaea *Halorhabdus rudnickae* and *Natrinema salaciae*

K. Krawczyk[1], *A. Bekier*[1], *M. S. da Costa*[2], *L. Albuquerque*[2], *M. Kowalewicz-Kulbat*[1];
[1]1Department of Immunology and Infectious Biology, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland, [2]Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal.

Introduction: The domain *Archaea* is one of the three domains of life. This domain comprises many extreme halophiles, defined as microorganisms that inhabit hypersaline environments. Halophilic archaea strains of *Halorhabdus rudnickae* WSM-64^T and WSM-66, were isolated from the hypersaline environment in Barycz mining area belonging to the Polish Salt Mine Company "Wieliczka". *Natrinema salaciae* strain MDB25^T was isolated from the deep, hypersaline anoxic Lake Medee in the Eastern Mediterranean Sea. The role of archaea as part of the human microbiome has been described but still remains unknown how halophiles can interact with the human cells. **Aim:** The aim of this study was to determine the effect of *Hrd. rudnickae* and *N. salaciae* on the phenotype of the human monocyte-derived dendritic cells (Mo-DC). **Materials and methods:** Monocytes were isolated from the human blood by MACS and incubated for 6 days with cytokines to generate Mo-DC. Mo-DC were stimulated with *N. salaciae* MDB25^T and *Hrd. rudnickae* WSM-64^T and WSM-66. The expression of CD86, CD80, HLA-DR, CD40 and DC-SIGN on the surface of Mo-DC was analyzed by flow cytometry. **Results:** We observed significantly decreased expression of CD80, HLA-DR and DC-SIGN receptors on the surface of *Hrd. rudnickae* WSM-64^T and WSM-66, and *N. salaciae* MDB25^T-stimulated Mo-DC, compared to unstimulated cells. There were no differences in the expression of CD40 and CD86. **Conclusion**: Our results suggest that halophiles possess the ability to diminish some signals in immune synapse what may have an impact on the naive T cell differentiation. Further research should provide insights into DC and T cell cytokine production.

P.A5.07 Initiation of immune responses - Part 7

P.A5.07.01

Type 1 hypersensitivity and reduced level of interferon-ã in cement loaders

M. O. Akiibinu[1], *T. Oduola*[2];
[1]Caleb University Lagos, Lagos, Nigeria, [2]Usmanu Danfodiyo University, Sokoto, Sokoto, Nigeria.

Introduction: Immunomodulatory potentials of cement particles have not been well researched in occupationally exposed Nigerians. This study was designed to investigate the possibility of type 1 hypersensitivity reaction and cellular activation in cement exposed workers. **Methods:** Twenty-nine male cement loaders who had direct exposure to cement dust and gases for a period of 2-30 years in Elephant / Lafarge Cement Depot Ibadan, Nigeria, were recruited for this study. Another twenty apparently healthy individuals who had no interaction with cement served as controls. Plasma immunoglobulin E (IgE) and interferon-gamma (IFN-γ) were determined in them using enzyme linked immunosorbent assay methods. **Results:** The results showed significantly ($p<0.05$) higher level of plasma IgE in cement loaders compared with controls. IFN-γ decreased significantly ($p<0.05$) in cement loaders compared with controls. There was no significant ($p>0.05$) correlation between the IgE, IFN-γ and period of exposure in the cement loaders. **Conclusion:** Cement dust could evoke IgE production and possibly inhibits certain cell types secreting IFN-γ in occupationally exposed workers.

P.A5.07.02

CD5signalosome coordinates TCR signals to control the generation of peripherallyinduced regulatory T cells

G. BLAIZE[1], *N. Rouquié*[1], *M. Marcellin*[2], *M. Benamar*[1], *A. Gonzalez de Peredo*[2], *O. Schiltz*[2], *R. Lesourne*[1];
[1]*CPTP InsermU1043 CNRSUMR5282, TOULOUSE, France,* [2]*IPBS UMR5089, TOULOUSE, France.*

Introduction: CD5 proteins are TCR co-receptors initially described as negative regulators of T cell signaling and T cell responses. Despite many studies performed mainly in cell lines, the molecular mechanisms mediated by CD5 on primary T cells remain unclear.
Results: We performed mass spectrometry (MS) analysis of CD5 partners in primary T cells. We identified a molecular complex recruited on CD5 upon TCR engagement. In this complex, the ubiquitin ligase Cbl is the most abundant protein. We identified Y429 of CD5 as a potential target of Cbl binding. We designed transgenic mice expressing the Wt CD5 ($CD5tg^{Wt}$) or a mutated form of CD5 on its Y429 ($CD5tg^{Y429F}$), in which we demonstrate by MS that the recruitment of all CD5 partners is impaired. In the context of CD5, we identified Cbl as an adaptor molecule allowing the recruitment of CD5 partners to the Y429 of CD5. Our results show that the molecular complex recruited on CD5 regulates both positively and negatively TCR signaling pathways and acts negatively on conventional T cell activation/proliferation and on iT_{reg} generation *in vitro* and *in vivo*.
Conclusion: In this work, we revisited CD5 mechanisms and functions by identifying Y429 of CD5 as a critical residue to recruite CD5 partners. Our work suggests that CD5 could optimize immune responses by setting the threshold for conventional T cell and pT_{reg} biological functions.

P.A5.07.04

Investigating the interaction and orientation of the immune cell proteins CD2, CD4 and CD45 on model membranes using hydrodynamic trapping

V. Junghans[1], *A. M. Santos*[2], *S. J. Davis*[2], *P. Jönsson*[1];
[1]*Lund University, Lund, Sweden,* [2]*University of Oxford, Oxford, United Kingdom.*

Different proteins play an important role during the cell-cell contact formation and are highly organized in respect to their size and function. However, crucial information about intermolecular interactions and height-dependent orientation of the proteins, especially the glycoprotein CD45, are lacking. We show by using hydrodynamic trapping (HDT) how these missing parameters for the immune-cell molecules CD2, CD4 and CD45 can be obtained[1].
In HDT a micropipette is positioned above a supported lipid bilayer (SLB) and negative pressure applied through the micropipette results in accumulation of the proteins attached to the SLB[2]. Relating the protein accumulation to the trapping strength both the molecular size/orientation as well as the intermolecular force between the proteins can be determined. In our system CD2 and CD4 oriented in an upright position from the bilayer, whereas CD45 had more freedom rotating relative to the surface. With increasing surface coverage, this flexibility reduced and CD45 positioned upright. Neither of the studied proteins aggregated or formed dimers but glycosylation had a high effect on the protein interaction. Trapping of two proteins simultaneously led to size-dependent protein segregation in the trap. Thus, the HDT method opens for new information about membrane protein behaviour and shows how protein flexibility and glycosylation can dominate this behaviour.
References:
1. Junghans, V., Hladilklova, J., Santos, A.M., Lund, M., Davis, S.J. Jönsson, P. Hydrodynamic trapping measures the interaction between membrane-anchored molecules. *Submitted* (2018)
2. Jönsson, P. et al. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 10328-33 (2012)

P.A5.07.05

Nanobodies from transgenic mice

T. Eden, S. Menzel, T. Schäfer, A. Eichhoff, F. Haag, ***F. Koch-Nolte****;*
University Medical Center Hamburg, Hamburg, Germany.

Llamas and other camelids carry a variant immunoglobulin locus that encodes antibodies composed only of heavy chains. The single variable domain of these antibodies (designated VHH or nanobody) has been shaped by evolution for high solubility and stability, independent of a partner VL domain. With their long CDR3s, nanobodies can reach hidden epitopes that are not accessible for conventional antibodies. Nanobodies can be used in monovalent format, e.g. for high resolution microscopy or as crystallization chaperones. Due to their high solubility and stability nanobodies can readily be fused to other proteins. For example, fusion to other nanobodies yields bispecific or multispecific reagents, fusion to the hinge-and Fc domains heavy chain antibodies of any desired isotype. In order to facilitate the generation of nanobodies for biomedical applications, we have generated nanobody-transgenic mice by transferring an engineered llama IgH locus to IgH-ko mice. Immunization of these mice induces antigen-specific heavy chain antibody responses with efficient VDJ recombination, somatic hypermutation, and class switch from IgM to IgG. These mice thus provide a flexible new platform for generating innovative nanobody-based biologics. They also provide a basis for genetic modification of nanobodies and the generation of designer nanobodies.

P.A5.07.06

MHC presentation is limited by the availability of MHC molecules rather than by the supply of peptide ligands

L. R. Komov*, D. Melamed Kadosh, E. Barnea, A. Admon;*
Technion-Israel Institute of Technology, Haifa, Israel.

Despite the importance of peptide presentation by the MHC class I, some aspects of the MHC processing and presentation are yet unsolved. One of those questions is whether the MHC presentation level is limited by the availability of peptide ligands within the ER or by the supply of peptide-receptive (empty) MHC molecules. Our study clarifies this issue by inducing major perturbations and competition for MHC ligands in human breast cancer cells (MCF-7). The cells were treated with interferons, which led to elevated presentation levels of the MHC-B molecules with their bound peptidome, relative to the MHC-A and MHC-C molecules of the same cells. This result was unexpected, since all of the MHC allotypes of the MCF-7 cell line present peptides with similar molecular properties; the treatments were expected to have similar effect on all of the MHC allotypes. Furthermore, high expression levels of recombinant soluble MHC-A were induced in the cells, to create a competition for peptides between the soluble and the identical endogenous membranal MHC-A. This competition did not affect the membranal MHC-A presentation levels or its bound peptidome. Our results suggest that in contrary to the common opinion, the MHC presentation levels are limited by the availability of peptide-receptive molecules rather than by the supply of peptides. These findings are important for the basic understanding of the antigen processing and presentation pathway, as well as vaccines design for pathogens infections and cancer immunotherapy. Supporting: the I-CORE Program of the Planning and Budgeting Committee and the Israel Science Foundation

P.A5.07.07

Specialized pro resolving mediators as novel therapeutic agents in treating neuroinflammation

A. Leuti[1,2], *E. Bisicchia*[3], *A. Cordella*[3,4], *V. Sasso*[3], *M. D'Amelio*[3,2], *N. Mercuri*[3,4], *M. Viscomi*[3], *V. Chiurchiù*[1,2];
[1]*Lipid Neurochemistry Unit, European Center for Brain Research (CERC), IRCCS Santa Lucia Foundation, Roma, Italy,* [2]*Department of Medicine, Campus Bio-Medico University of Rome, Rome, Italy,* [3]*1IRCCS Santa Lucia Foundation, Roma, Italy,* [4]*Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy.*

Specialized pro resolving mediators (SPMs) are a novel class of endogenous lipids, produced by innate immune cells from essential omega-3 polyunsaturated fatty acids, that orchestrate the resolution of inflammation (i.e. the spontaneous confinement and regression of the inflammatory wave started by the immune response) and promote tissue regeneration. SPMs are emerging as crucial players in several central and peripheral diseases caused by chronic and unconfined inflammation. Herein, we investigated the involvement and therapeutic outcome of a specific SPM, termed resolvin D1 (RvD1), in two neuropathological paradigms, namely a rat model of remote and focal brain damage, obtained through hemicerebellectomy (HCb), and in a transgenic alpha-synuclein (a-Syn) overexpressing rat model of Parkinson's disease, for both of which neuroinflammation is a key hallmark. HCb-injured animals displayed reduced levels of RvD1 in cerebrospinal fluid (CSF) and unchanged levels in plasma, whereas such SPM was increased in CSF and decreased in plasma of α-Syn rats at an early stage of the disease characterized by a precocious neuroinflammation, dopaminergic and motor dysfunctions but absence of dopaminergic neuronal loss. Chronic administration of RvD1 in HCb reduced glial activation and blocked inflammatory-induced neuronal cell death in remote regions. Similarly, early and chronic 2 months treatment in a-Syn rats halted neuroinflammation, improved striatal dopaminergic synaptic function and improved motor-behaviour functions. Overall, our findings strongly suggest that, not only the uncontrolled inflammation observed in these models can be ascribed to an impaired SPM-dependent resolution machinery, but also, that specific resolvins represent novel and safe therapeutics for neuroinflammatory brain diseases.

P.A5.07.08

Inhibition of TLR and TLR+BCR dependent functions of human B cells by Complement Receptor Type 1 (CD35)

B. Mácsik-Valent*;*
Department of Immunology, Budapest, Hungary.

Although it is well accepted that separate activation of the complement system and Toll-like receptors (TLRs) initiates and shapes the adaptive immune response, much less is known about the modulation of various B cell functions by the simultaneous activation of these two systems. Therefore we investigated how engagement of complement receptor type 1 (CR1) influences the activation of human B cells induced by TLR7 and TLR9 with or without a BCR stimulus. Resting tonsillar B cells were activated via BCR by a suboptimal dose of $F(ab')_2$ anti-human IgG/M/A and via TLR7 and TLR9 by synthetic activators. The stimuli were applied either separately or simultaneously in the presence or absence of the CR1 ligand, a multimeric "C3b-like C3". The effect of CR1 clustering was assessed on proliferation (^{3}H-thymidine incorporation), cytokine secretion (ELISA), antibody production (ELISPOT) and expression of activation markers (flow cytometry).

We show that CR1 clustering significantly and dose dependently reduces the TLR9-induced activation of tonsillar B cells, but has no effect on the TLR7-induced functions. The enhanced response to the simultaneous engagement of TLR7 or TLR9 with the BCR was also significantly inhibited by CR1 clustering. Our data demondtrate that engagement of CR1 downregulates the TLR9-induced B cell functions but does not influence the TLR7 mediated processes. Interestingly however, when B cells are simultaneously triggered via BCR+TLR7 or BCR+TLR9, CR1 clustering inhibits the B cell response. We assume that CR1 exerts its inhibitory effect by acting on signalling molecules linked to both BCR and TLR9 in human B cells.

P.A5.07.09
c-Myc in T lymphocytes: How is it controlled? What does it control?

***J. M. Marchingo**, L. V. Sinclair, D. A. Cantrell;*
Cell Signalling and Immunology Division, School of Life Sciences, The University of Dundee, Dundee, United Kingdom.

T cells undergo massive cell growth, rapid proliferation and differentiation to form a protective immune response. The proto-oncogenic transcription factor c-Myc plays a critical role in this process. To explore c-Myc function in T cells we used high-resolution mass spectrometry to compare the effect of c-Myc deficiency on the global proteome of antigen receptor-stimulated CD4 and CD8 T cells. Using this technology we quantified the expression of ~6700 proteins. c-Myc-deficiency reduced the copy number of ~4,400 and 2,400 proteins in CD8 and CD4 T cells respectively. The magnitude of c-Myc effect on individual proteins was selective. For example, while the amino acid transporter Slc7a5 was decreased 40 and 30-fold in CD8 and CD4 T cells, the glucose transporter Glut3 was 2.1 and 2.9-fold higher in the c-Myc-deficient CD8 and CD4 T cells respectively. In contrast Glut1 was the same across all conditions.
Systems with substantial biosynthetic defects can confound our ability to distinguish which proteins become dysregulated first and whether the effects are direct. For example Slc7a5 and c-Myc are both reported to regulate the other's expression in T cells but deficiency in either also substantially compromises RNA and protein biosynthesis. We investigated how nutrient availability and transporter levels interplayed with c-Myc expression. We found that c-Myc induction required extrinsic amino acids, but that Slc7a5 deficiency did not alter c-Myc induction; however it did compromise maintenance of c-Myc levels. Thus, c-Myc initiation of Slc7a5 triggers a positive feedback loop to maintain expression of both proteins and drive T cell growth.

P.A5.07.10
sFasL mediates proinflammatory activation of neutrophils from type 2 diabetes mellitus without affecting apoptosis

***S. Margaryan**[1,2], A. Witkowicz[3], A. Martirosyan[2], A. Partyka[3], L. Karabon[3], G. Manukyan[1,2];*
[1]Russian-Armenian University, Yerevan, Armenia, [2]Institute of Molecular Biology NAS RA, Yerevan, Armenia, [3]L. Hirszfeld Institute of Immunology and Experimental Therapy, Wroclaw, Poland.

Introduction: Type 2 diabetes mellitus (T2DM) is a chronic metabolic condition characterized by insulin resistance and pancreatic β-cell dysfunction. Immune cell activation associated with persistent low-grade inflammation play a prominent role in apoptosis-mediated β-cell destruction and vascular injury in T2DM. Despite Fas/FasL axis was implicated in the development of diabetes-related complications, the role of soluble FasL (sFasL) is still unknown. Therefore, we aimed to analyze the influence of sFasL on neutrophil activation and apoptosis *in vitro*. Methods: For this, transcriptional and expression levels of pro-inflammatory and apoptotic genes were measured in neutrophils from T2DM patients (n=16) and healthy controls (n=15) exposed with sFasL for 3 hours. Results: sFasL significantly increased mRNA levels of NF-κB, IL-1β, caspase-1 in neutrophils isolated from T2DM patients which was associated with the increased CD18 MFI. At the same time, apoptotic rates of the cells from both studied groups were unaffected by sFasL, which was accompanied by the unchanged mRNA levels of Bax, decreased mRNA levels of caspase-3 and decreased number of Fas (CD95) positive neutrophils. In T2DM, the presence of sFasL significantly increased production of IL-8 by whole blood cells compared to both control cultivation and sFasL-induced healthy cells. Conclusion: Thus, we showed an enhanced inflammatory response of neutrophils from T2DM patients to sFasL without acceleration of apoptosis, which may play an important role in induction and/or sustaining of inflammation in the disease.

P.A5.07.11
The 20s immunoproteasome and the constitutive proteasome bind with the same affinity to PA28αβ and equally degrade FAT10

***n. roverato**;*
university of konstanz, konstanz, Germany.

The 20S immunoproteasome (IP) is an interferon(IFN)-γ - and tumor necrosis factor (TNF) -inducible variant of the 20S constitutive proteasome (CP) in which all its peptidolytically active subunits β1, β2, and β5 are replaced by their cytokine inducible homologues β1i (LMP2), β2i (MECL-1), and β5i (LMP7). These subunit replacements alter the cleavage specificity of the proteasome and the spectrum of proteasome-generated peptide ligands of MHC class I molecules. In addition to antigen processing, the IP has recently been shown to serve unique functions in the generation of pro-inflammatory T helper cell subtypes and cytokines as well as in the pathogenesis of autoimmune diseases, but the mechanistic involvement of the IP in these processes has remained elusive. In this study we investigated whether the IP differs from the CP in the interaction with two IFN-γ/TNF inducible factors: the 11S proteasome regulator PA28αβ and the ubiquitin-like modifier FAT10 (ubiquitin D).

P.A5.07.12
Progesterone suppresses the inflammatory state of innate cells in peripheral blood and cervical mucous

***G. R. Sooranna**, N. M. Shah, A. Cocker, N. Singh, M. R. Johnson;*
Imperial College London, London, United Kingdom.

Introduction: Progesterone (P4) has been shown to be an effective immune-modulator and, in reproductive tissue, P4 suppresses inflammation and maintains uterine quiescence. In clinical practice, P4 supplementation is an effective treatment for the prevention of preterm birth (PTB). Our hypothesis was that P4 skews innate cell phenotype systemically in peripheral blood and locally in cervical mucous, to attenuate inflammatory responses. Method: We recruited pregnant patients (N=6) between 13-20 weeks of gestation identified as high risk for developing PTB and thus commenced on P4 treatment. Peripheral blood (PB) and cervical brush samples (Cb) were collected before and one month after starting P4. Flow-cytometric analysis of PB and Cb mononuclear cells (PBMC and CbMC) was performed. All p-values were two-tailed and unadjusted and significance was defined as $p < 0.05$. Results: P4 treated women (n=6) showed a significant decrease in peripheral blood classical monocytes (CD14+CD16-; p=0.0218) but stable granulocyte proportions. In addition, post P4 treatment CbMC showed a decrease in total neutrophils (p=0.0456), and an increase in apoptotic CD14+ neutrophils (p=0.0313). However, the expression of HLA-DR and CD66b on cervical neutrophils was either unaffected or increased, and this was accompanied by a trend suggesting an increased proportion of macrophages. Conclusion: P4 treatment is associated with attenuation of inflammatory innate cell phenotype, which likely reflects the overall anti-inflammatory effect of P4. Despite these effects on the cervix, the immune-protective potential of cervical mucous does not appear to be hampered.

P.A5.07.13
Functional CD169 on macrophages mediates interaction with dendritic cells for CD8$^+$ T cell cross-priming

***D. van Dinther**[1], H. Veninga[1], S. Iborra[2], E. G. Borg[1], L. Hoogterp[1], K. Olesek[1], M. R. Beijer[1], H. Kalay[1], J. Garcia-Vallejo[1], K. L. Franken[3], L. B. Cham[4], K. S. Lang[4], Y. van Kooyk[1], D. Sancho[2], P. R. Crocker[5], J. M. den Haan[1];*
[1]VUmc Amsterdam, Amsterdam, Netherlands, [2]Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain, [3]LUMC, Leiden, Netherlands, [4]University Duisburg-Essen, Essen, Germany, [5]University of Dundee, Dundee, United Kingdom.

Splenic CD169$^+$ macrophages are located in the marginal zone to efficiently capture blood-borne pathogens. Here, we investigate the requirements for the induction of CD8$^+$ T cell responses by antigens (Ags) bound by CD169$^+$ macrophages. Upon Ag targeting to CD169$^+$ macrophages, we show that Batf3-dependent CD8α$^+$ dendritic cells (DCs) are crucial for DNGR-1-mediated cross-priming of CD8$^+$ T cell responses. In addition, we demonstrate that CD169, a sialic acid binding lectin involved in cell-cell contact, preferentially binds to CD8α$^+$ DCs and that Ag transfer to CD8α$^+$ DCs and subsequent T cell activation is dependent on the sialic acid-binding capacity of CD169. Finally, CD8+ T cell responses to vaccinia virus infection are dependent on functional CD169. Together, these data indicate that the collaboration of CD169$^+$ macrophages and CD8α$^+$ DCs for the initiation of effective CD8$^+$ T cell responses is facilitated by binding of CD169 to sialic acid containing ligands on CD8α$^+$ DCs.

P.A5.07.14
Modulation of Th17 response in cultures of human peripheral blood mononuclear cells

***P. Vidović**[1], S. Tomić[2,3], M. Bekić[2,3], M. Milanovic[2], S. Rakočević[1], D. Vučević[2], M. Čolić[1,2,3];*
[1]University of East Sarajevo, Medical Faculty in Foca, Foča, Bosnia and Herzegovina, [2]University of Defense in Belgrade, Medical Faculty of the Military Medical Academy, Belgrade, Serbia, [3]University of Belgrade, Institute for Application of Nuclear Energy, Belgrade, Serbia.

Numerous studies performed on purified CD4$^+$ T cells have shown that the cytokine cocktail (IL-1β/IL-6/IL-23) is required for Th17 cell polarization, whereas the role of TGF-β remains controversial. We examined how Th17 response is modulated in the whole peripheral blood mononuclear cell (PBMNC) cultures, which better reflect conditions *in vivo.* PBMNC were isolated from healthy volunteers. The cells were cultivated under different conditions in the presence of IL-1β/IL-6/IL-23, with or without TGF-β. For stimulation, phytohemaglutinine (PHA) or CD3/CD28 antibodies were used. Some cultures were treated with either IL-10, TGF-β or IFN-γ neutrilizing antibodies.

Our results indicated that in PHA- and CD3/CD28-stimulated PBMNC cultures the production of IL-17 was significantly higher (670pg/mL and 930 pg/mL), respectively, compared to unstimulated cultures (48pg/mL) and this finding correlated with the number of cells. The IL-1β/IL-6/IL-23 cocktail significantly augmented the production of IL-17 in unstimulated and CD3/CD28-stimulated PBMNC cultures, but not in PHA-stimulated cultures. The addition of TGF-β to the cytokine cocktail inhibited the production of IL-17 in all three culture systems. The opposite effect was seen when anti-TGF-β or anti-IFN-γ antibodies were added. It is interesting that anti-IL-10 antibody augmented the percentage of IL-17$^+$ cells, but the production of IL-17 was reduced in the supernatants. In conclusion, our results showed that IL-1β/IL-6/IL-23 stimulated differentiation and activation of Th17 cells in PBMNC cultures, but the effect depended on the type of T-cell activation stimuli. The Th17 response was better when TGF-β and Th1-signaling pathways were inhibited. However, the role of IL-10 signaling pathway remains unclear.

P.A5.07.15

Complement factors F, B, H and schizophrenia in Armenians

R. Zakharyan[1,2]*, H. Ghazaryan*[2]*, A. Arakelyan*[1,2]*;*
[1]*Russian-Armenian University, Yerevan, Armenia,* [2]*Institute of Molecular Biology NAS RA, Yerevan, Armenia.*

Immune system alterations contributed to schizophrenia. We aimed to assess the blood levels of complement factors F (FB) and FH in schizophrenia. According to the results, the FB serum levels were 1.3 times decreased in patients compared to controls (meanSD,ug/mL: 220.061.04 versus 276.865.46, p<0.01). No significant difference in the mean FB between antipsychotic-free and treated patients was detected. This reduction may reflect the increased activity of alternative pathway and consequent increased FB degradation. The FH levels were equal in study groups. Further evaluation of the total FH, its 402H and 402Y variants (Y402H, rs1061170) in patients and controls was performed. We found no significant difference in the total FH levels between groups (265.6±28.4 vs 241.9±52.1, p>0.5). Also, there was no difference in total FH between 402H minor variant carriers (YH+HH) and standard variant (YY) homozygotes (235.9±49.2 vs 247.4±56.1, p>0.5) and between the groups (270.9±44.2 vs 286.5±95.6, p>0.5). In controls the 402H level tended to be lower in YH+HH than in YY (255±24.4 vs 283.2±27.3, p=0.05). In patients the 402Y levels were lower in YH+HH (71.92±57.9 vs 216.2±79.9, p<0.0001). The 402H levels in patients were marginally lower compared to 402Y level (89.6±110.4 vs 144.1±100.5, p=0.06), whereas a significantly increased 402H level compared to 402Y in controls was observed (265.6±28.4 vs 79.6±71.9, p<0.0001). These results suggested the important role of FB and FH in schizophrenia. Further replication studies are needed to confirm these findings.

P.A5.07.16

Development of novel tools to investigate trogocytosis in T lymphocytes

S. Zenke[1]*, J. Braun*[1]*, S. Ammann*[2]*, N. Beyersdorf*[3]*, P. Aichele*[4]*, G. Griffiths*[2]*, J. Rohr*[1]*;*
[1]*Center for Chronic Immunodeficiency, Freiburg, Germany,* [2]*Cambridge Institute for Medical Research, Cambridge, United Kingdom,* [3]*Institute for Virology and Immunobiology, Würzburg, Germany,* [4]*Institute of Immunology, University Medical Center Freiburg, Freiburg, Germany.*

T cells have long been known to extract surface molecules from antigen-presenting cells – a process termed “trogocytosis”. However, the molecular requirements and biological function of this process is incompletely understood. In order to investigate the mechanisms and function of trogocytosis in T cells we generated cell lines stably expressing an antigenic peptide covalently linked to a blue fluorescent pMHC-complex in addition to red fluorescent B7-molecules. Using these artificial antigen presenting cells (APCs) we find that naïve CD8+ T cells rapidly and efficiently acquire peptide-MHC complexes and B7 molecules from artificial APCs. This process is dependent on specific interactions between the T cell receptor/peptide-MHC and CD28/B7. Transfer of B7 does not require specific TCR engagement, but concomitant pMHC-recognition increases the efficacy of B7 transfer. Furthermore, we show that a substantial part of trogocytosed molecules can be re-expressed on the T cell surface – a finding that we confirmed to also occur in murine models *in vivo*. Currently, we are interfering with specific cellular function to reveal the molecular mechanisms underlying trogocytosis in T cells. In summary, trogocytosis constitutes a mechanism how T cells can efficiently acquire and re-express surface molecules from neighbouring cells – thereby equipping them with the armamentarium of bona-fide APCs.
Funded by DFG-SFB1160

P.A5.07.17

Low molecular weight hydrogels as injectable scaffold for tuning the foreign body reaction

S. Kotagudda ranganath[1]*, N. Bansode*[2]*, C. Boiziau,*[1]*, B. Hinz*[3]*, S. Rey*[1]*, l. Laxtague*[2]*, N. mano*[4]*, A. kuhn*[5]*, P. Barthelemy*[2]*, O. Chassande*[1]*;*
[1]*Inserm ,Biotis U-1026, Bordeaux, France,* [2]*Chembiomed, INSERM U 869, Bordeaux, France,* [3]*Laboratory of Tissue Repair and Regeneration, Matrix Dynamics Group, University of Toronto, Toronto, Canada,* [4]*Biofuel cell team CRPP, CNRS, Pessac, Bordeaux, France,* [5]*Analytical Nanosystems group, ISM, Talence, Bordeaux, France.*

Introduction: One of the major challenges for the use of implantable biomaterials is to develop strategies that moderate the innate host inflammatory reaction. Among biomaterials that are considered for clinical applications, hydrogels have gained a significant interest due to their tunable mechanical properties. However, only few are used in clinics because hydrogels, like other biomaterials, induce FBRs after implantation. The severity of the hydrogel-induced FBR is different for each biomaterial. To identify modifications that suppress the FBR, we developed four novel low molecular weight supramolecular hydrogels that were tested and compared in a mouse model of subcutaneous implantation.
Methods: Subcutaneous implantation of hydrogels was performed in mice to assess different aspects of the FBR. The type of inflammatory cells in the surrounding tissue, as well as within the hydrogels, was determined by histology. Specific markers for angiogenesis, macrophage polarisation, and fibroblast were labeled to assess the FBR. Mass spectrometry was used to characterize degradation products of the hydrogel biomaterial.
Results and Conclusion: Our results indicate that all novel low molecular weight hydrogels showed low inflammatory response by modulating the macrophage polarisation, enhancing vascularisation in the surrounding tissue, and absence of fibrous capsule formation. A potential angiogenic property of the degradation product is currently under investigation. The results indicate that hydrogels are potential Biomaterial candidate for various application such as tissue engineering and medical devices.
Keywords: Foreign body reaction, macrophages, inflammation, fibrosis, hydrogels.
Acknowledgment: The project is funded from the Laboratory of Excellence AMADEus with the reference ANR-10-LABX-0042-AMADEUS.

P.A5.07.18

Metabolic mimicry of regular high-intensity exercise modulates neutrophil function.

J. Sanchez*, S. Walker, A. Gutierrez del Arroyo, G. L. Ackland;*
Queen Mary University of London, London, United Kingdom.

Introduction
Regular high-intensity exercise is associated with longevity and improved health. Modulation of innate immunity may contribute to the positive impact of exercise, although the mechanisms remain unclear. High-intensity exercise is characterised by accumulation of lactate as a product of anaerobic glycolysis. We examined whether mimicking repeated high-intensity exercise through direct, repeated exogenous administration of lactate may modulate innate immune responses.
Methods: C57BL/6 mice were randomised to receive injected intraperitoneal (200µl) injections of either saline or sodium-lactate to mimic high-intensity exercise on 5 consecutive days. 24h after the last pre-treatment, zymosan (1mg) was injected to elicit neutrophil influx into the peritoneum. Peritoneal lavage and bone marrow were harvested either 2.5h or 5h later. Neutrophil CD11b expression and ex-vivo phagocytosis of *Staphylococcus aureus* bioparticles by peritoneal neutrophils were assessed by flow cytometry.
Results: More CD45$^+$CD11b$^+$Ly6G$^+$ cells (11.05±2.37x10^6/ml) were present in the peritoneum after sodium-lactate pre-treatment, compared to saline (3.96±1.13x10^6/ml; n=11/group, p=0.02) after 2.5h of zymosan injection. Sodium-lactate pre-treatment was associated with a reduction in peritoneal neutrophil CD11b expression (median fluorescence intensity (MFI): 3277±642), compared to saline pre-treatment (MFI: 7662±1207; n=7, p=0.008) after 5h of zymosan injection. However, bone-marrow resident neutrophils had similar CD11b expression, irrespective of pre-treatment (p=0.85). Sodium-lactate pre-treatment augmented ex-vivo phagocytosis of *S. aureus* by 848 bioparticles (95% confidence intervals: -49 to 1744; n=5, p=0.06).
Conclusion: Mimicking high-intensity exercise by exogenous pre-treatment with sodium-lactate modulates the neutrophil response to acute inflammation.

P.A5.07.19

Changes in the T cell receptor repertoire during treatment interruption in paediatric patients with HIV

T. C. Attenborough[1]*, K. Schou Sandgaard*[1]*, B. Margetts*[1]*, S. Adams*[2]*, R. Callard*[1]*, A. Gkazi Soragia*[1]*, N. Klein*[1]*;*
[1]*UCL Great Ormond Street Institute of Child Health, London, United Kingdom,* [2]*Great Ormond Street Hospital for Children, London, United Kingdom.*

Antiretroviral therapy (ART) is generally very effective in children infected with HIV, and they can often recover from the depletion of CD4$^+$ T cells caused by the disease. However side effects can sometimes lead to medication nonadherence or medical recommendation for a treatment break. Studies have already shown that CD4$^+$ cell levels are generally restored with ART reintroduction. This study used Next Generation Sequencing (NGS) techniques to examine the impact of ART interruption and reintroduction on the T cell receptor (TCR) repertoire in great detail.
We used NGS to estimate the TCR repertoire from 4 paediatric patients living with HIV. The samples were accessed from a randomised controlled trial and span before, during, and after a 48 week ART treatment interruption. The samples were purified to collect the naive CD4$^+$ T cells and memory CD8$^+$ T cells from each sample.
We found similarities in the TCR repertoire profiles before the ART interruption and post reintroduction. We were able to track specific CDR3 sequences over time. A large number of CDR3 sequences were also shared between the patients, and showed several different patterns over the time.
The similarity of the TCR repertoire profiles at the beginning and end of the study suggests that in general, the treatment interruption in paediatric patients doesn’t appear to have long term negative effects on the TCR repertoire. We also found CDR3 sequences in both naive CD4$^+$ T cells and memory CD8$^+$ T cells that were shared between patients.

P.A6.01 Lessons learned from the genetic defects - Part 1

P.A6.01.01
The effect of PI3K pathway in response to TLR stimulation of Rendu-Osler-Weber syndrome patient

N. Bozbeyoglu[1], *G. G. Kaya*[1], *M. Yildirim*[1], *I. Evcili*[1], *I. C. Ayanoglu*[2], *O. Ardeniz*[3], *M. Gursel*[2], *I. Gursel*[1];
[1]*Bilkent University, Ankara, Turkey,* [2]*Middle East Technical University, Ankara, Turkey,* [3]*Ege University, Izmir, Turkey.*

Rendu-Osler-Weber syndrome is a rare autosomal dominant disease which is characterized by lesions occuring due to vascular enlargement in the skin and mucosa. Additionally, Common Variable Immunodeficiency (CVID) is the most common primary immunodeficiency that is manifested by recurrent infections and inflammations, although symptoms of individuals are variable. In this study, due to the possibility of differences in PI3K/AKT/mTOR pathway, stimulation of TLR pathways, gene expression and plasma cytokine levels are examined from peripheral blood of Rendu-Osler-Weber (ROW) patient. Isolated PBMC was stimulated with PMA/Ionomycin, CD3/CD28, LPS, CpG ODNs (K- and D-types). The IL-4, IL-8, IL-10, IL-17 and IFN-γ levels were measured by ELISA. It was observed that when stimulated with PMA/Ionomycin and CD3/CD28 ligands, IFN-γ level did not differ compared to healthy measurements, whereas LPS induced significant increase. When PBMCs were stimulated with PMA/Ionomycin, IL-4, IL-10, IL-17 levels were lower than healthy PBMC levels. Plasma cytokine levels were measured by CBA. IFN-γ and IL-10 levels were significantly higher than healthy levels. The inflammation panel analyses via Nanostring™ revealed that TLR2, TLR4, TLR7/8, NF-κB, Stat1, IL-15 levels were magnified compared to healthy PBMCs. PI3K pathway analyses implicated that RTK gene level was increased, whereas mRNA levels of Ras, PKCs, ERK were decreased in ROW patient. There was insufficient data for PI3K and PTEN mRNA levels. The chemokine network analyses suggested an increased expression of STATs. Strikingly, chemokine receptors were decreased. Our investigation implied that ROW patients responses could be worsen during ongoing active infection.

P.A6.01.02
Late onset of maternal CD8 T-cells GVHD in a toddler with JAK3 severe combined immunodeficiency

R. de la Varga Martínez[1], *J. M. Lucena Soto*[1], *W. Goycochea-Valdivia*[2], *P. Sánchez-Moreno*[2], *P. Olbrich*[2], *B. de Felipe*[2], *B. Sánchez*[1], *O. Neth*[2];
[1]*Servicio de Inmunología. UGC de Laboratorios Clínicos. Hospital Universitario Virgen del Rocío, Sevilla, Spain,* [2]*Paediatric Infectious Diseases, Rheumatology and Inmunology Unit, Hospital Universitario Virgen del Rocío, Instituto de Biomedicina de Sevilla, Sevilla, Spain.*

Introduction: Autosomal recessive severe combined immunodeficiency (SCID) due to Janus kinase (JAK) 3 deficiency is characterized by a T-B+NK- phenotype. Objective: To describe a delayed presentation of maternal CD8 engraftment in a 15-month-old boy associated with fever, failure to thrive, hepatomegaly and atopic dermatitis. Prior to his referral he had persistent CMV viremia (PCR max 1500c/ml) not receiving antiviral treatment and MMR vaccine given aged 12 months was not associated with complications. Family history revealed parental consanguinity and a sister died at 7 months due to infectious complications. Material and methods: Immunophenotype using flow cytometry in peripheral blood, quantification of immunoglobulins and next generation sequencing of the genes related to SCID were performed. Results: Lymphocyte subsets showed absent levels of CD4+ T-cells, decreased NK-cells, normal CD8+ T-cells and elevated B-lymphocytes. IgG and IgM levels were normal for age (IgA absent). T-cell receptor excision circles (TRECS) and kappa-deleting recombination excision circles (KRECS) of the dried blood sample were 0/punch and 31/punch (normal >6 and >4/punch, respectively). Maternal CD8+ engraftment was confirmed using HLA-DR typing. The variant c.2892G>C (p.Lys964Asn), not previously described, was found to be homozygo in the JAK3 gene and confirmed by Sanger sequencing. Conclusions: We describe a child with a combined immunodeficiency and late onset of maternal CD8+ GVHD due to a new homozygous mutation in JAK3. Interesting the toddler did not suffer from disseminated CMV infection nor live vaccine associated complications to be expected in this immunocompromised host, probably due to the "protective" effect of maternal CD8 cells.

P.A6.01.03
A novel splicing mutation in a patient with Griscelli syndrome type 2

R. de la Varga Martínez[1], *M. López-Marcos*[2], *A. Pérez-Sánchez*[3], *P. Olbrich*[2], *B. Sánchez*[1], *O. Neth*[2], *J. M. Lucena Soto*[1];
[1]*Servicio de Inmunología. UGC de Laboratorios Clínicos. Hospital Universitario Virgen del Rocío, Sevilla, Spain,* [2]*Paediatric Infectious Diseases, Rheumatology and Inmunology Unit, Hospital Universitario Virgen del Rocío, Instituto de Biomedicina de Sevilla, Sevilla, Spain,* [3]*Department of Neonatología. Hospital Universitario Virgen del Rocío, Sevilla, Spain.*

Introduction: Griscelli syndrome type 2 (GS2, OMIM 214450) is a rare disease of autosomal recessive inheritance. It is characterized by hypomelanosis with immunologic abnormalities with or without neurologic impairment, is caused by mutation in the RAB27A gene. Objective: To describe a novel splicing mutation in a patient with GS2 in a newborn with silver hair, hepatosplenomegaly, pancytopenia and hemophagocytic lymphohistiocytosis (HLH). Material and methods: A newborn 13-day-old female was referred to the immunology department for suspicion of SG2. Quantification of soluble IL-2 receptor (sIL2R) and Sanger sequencing (3-8 exons and adjacent intronic regions of the RAB27A gene) was performed. Results: The patient met diagnostic criteria for HLH (6/8): persistent fever, splenomegaly, cytopenias (haemoglobin 92 g/L, platelets $9x10^9$/L, neutrophils $0.0x10^9$/L), low levels of fibrinogen (0.73 g/L), raised levels of ferritin (18107 µg/L) and sIL2R (118.39 ng/ml [0-7.5]). Due to the clinical instability BMA was not performed and NK cell activity studies were not available. The presence of the variant g.IVS6+1G>C is detected in homozygous at position g.67589663 of intron 6 of the RAB27A gene. These change causes an anomalous processing of the mRNA of the gene. Conclusions: This variant is described as polymorphism: rs756071120, found in heterozygous in a healthy control. However, we consider this to be a pathogenic mutation as the mutation affects an invariant dinucleotide splicing site, is in homozygous and the analysis of the mRNA shows anomalous processing. The results confirm the suspected SG2.

P.A6.01.04
Influence of an osteolysis predisposing allele TNF-238*A on the cytokine expression pattern of blood cells from total hip arthroplasty (THA) patients with mild and severe osteolysis

R. Fillerova[1], ***T. Dyskova***[1], *P. Schneiderova*[1], *V. Smotkova-Kraiczova*[1], *J. Gallo*[2], *E. Kriegova*[1];
[1]*Dept. of Immunology, Palacky University, Olomouc, Czech Republic,* [2]*Dept. of Orthopaedics, Palacky University&University Hospital, Olomouc, Czech Republic.*

Introduction: Individual susceptibility to periprosthetic osteolysis (PPOL) around total hip arthroplasty (THA) is associated with genetic variations in cytokine genes. Thus, cytokine pattern may differ in THA patients with mild and severe PPOL and in patients carrying a risk allele TNF-238*A, associated with severe osteolysis. Methods: Peripheral blood mononuclear cells (PBMCs) obtained from 31 THA patients with severe (n=23) and mild (n=8) osteolysis carrying (n=12)/non-carrying (n=19) a TNF-238*A risk allele were stimulated for 12h by lipopolysaccharide (LPS). Conditioned media were collected and protein concentrations of cytokines IL-2, IL-5, IL-10, IFN-γ, TNF-α, VEGF, and RANKL were measured by Luminex and ELISA. Results: Elevated expression of IL-2, IL-5, VEGF, and RANKL ($P<0.05$) was observed in supernatants from patients with severe comparing to mild osteolysis, irrespective of TNF-238 genotype. Patients with TNF-238 GG genotype with severe osteolysis showed up-regulated expression of all studied cytokines, except IL-10 and VEGF, comparing to those with mild osteolysis. In severe osteolysis, the carriers of rare TNF-238*A allele showed lower expression of IL-2, IFN-γ, TNF-α, and RANKL ($P<0.05$) when comparing to non-carriers. Conclusion: Alteration in cytokine expression pattern in LPS-stimulated PBMCs from THA patients with mild and severe osteolysis and differing by TNF-238*A genotype was observed. Molecular mechanisms by which TNF-238*A allele increases a risk of severe osteolysis should be further investigated. Grant support: MZ CR VES16-31852A, MZ ČR VES15-27726A, IGA UP_2018_016, MH CZ - DRO (FNOL, 00098892)

P.A6.01.05
TLR Dependent Immune Responses of Patient Displaying Common Variable Immunodeficiency Like Symptoms

I. Evcili[1], *G. G. Kaya*[1], *M. Yıldırım*[1], *N. Bozbeyoglu*[1], *I. C. Ayanoglu*[2], *Ö. Ardeniz*[3], *M. Gürsel*[2], *I. Gürsel*[1];
[1]*Bilkent University, Ankara, Turkey,* [2]*Middle East Technical University, Ankara, Turkey,* [3]*Ege University Faculty of Medicine, Izmir, Turkey.*

The innate immune system uses germline coded receptors to detect pathogens and mount acute innate response that helps to protect host from infection. Toll-like receptors (TLRs) and other pathogen dependent pattern recognition receptors play important role in this response. Common Variable Immunodeficiency (CVID) is the most common primer immunodeficiency seen in adults. Vitamin D and VDR have been reported to have an effect on TLR2. In this study, PBMCs from CVID-susceptible VDR+ patient was treated with TLR and Inflammasome ligands (Pam3CSK4, P (I: C), LPS, R848, Resiquimod, CpG ODNs, Nigericin and ATP) for 24h. IFNγ, IL-4, IL-8, Pan-IFNα and IP-10 levels were determined from the supernatants by ELISA. Total RNA isolated from PBMCs was studied with Nanostring™ inflammation panel. Th1/Th2/Th17 phenotyping was performed by flow cytometry and IP-10 and IL-1β levels in plasmas were determined by CBA. Both Nanostring™ and CBA analyses revealed that patient plasma had elevated IP-10 level. In addition, Nanostring implicated that patient's TLR3 and TLR7/8 mRNA levels were lower than healthy PBMCs. Lower pan-IFNα and IFNγ and higher IL-8 secretions in response to p(I:C), R848 and Resiquimod stimulation were observed compared to healthy controls. Similarly, TLR9 ligand stimulation gave lower pan-IFNα than healthy subject PBMCs. As a result, modulation of TLR pathways may offer new treatment options for CVID patients.

P.A6.01.06

Simple sequence repeat controls sex-dependent susceptibility to autoimmunity

***G. Fernandez Lahore**, M. Förster, M. Johannesson, R. Holmdahl;*
Karolinska Institutet, Solna, Sweden.

Complex autoimmune diseases have a strong genetic component. While genome-wide association studies have been very successful at identifying genetic determinants of large effect size, these studies run into limitations both in the identification of low effect size variants, and in the functional interpretation of identified non-coding variants. This creates a gap of unexplained genetic contribution has been termed *missing heritability*. Mouse genetic studies aid in the search for new genetic determinants by offering stable experimental conditions and the possibility for functional characterization. In this study we identified a non-coding simple repeat that reduced disease severity in mouse models of arthritis, multiple sclerosis, and delayed-type hypersensitivity in a sex-specific manner. We found that the simple repeat interfered with DNA-binding of the transcriptional regulator CTCF by creating a base pair change AC to GG within the CTCF consensus motif.
As a consequence, it affected the expression profile of nearby genes, including the TCR co-stimulator *Cd2*, changing the reactivity of peripheral T cells. We found that these changes in expression were specific to female mice, suggesting an involvement of sex hormones. Indeed, castration of female mice both regulated binding of CTCF to the candidate site, and was sufficient to reverse the protective effect in the EAE model of multiple sclerosis. In conclusion, we identified a non-coding simple repeat that dictates the expression profile of surrounding genes in a sex-dependent manner, culminating in a sexually dimorphic autoimmune phenotype.

P.A6.01.07

Genetic study in a spanish cohort of Narcolepsy type 1 patients and susceptibility to autoimmune diseases

***M. Fernández-Arquero**, B. González Fernández, L. N. Campo Blázquez, E. Rodríguez, I. Villalibre-Valderrey, S. Sánchez-Ramón, F. J. Martínez-Orozco;*
San Carlos University Hospital, Madrid, Spain.

Introduction. Narcolepsy Type 1 (NT1) affects between 0.025-0.40% population in Europe. This chronic rare sleep disorder is characterised by excessive daytime sleepiness, cataplexy and disturbed nocturnal sleep. It is caused by a selective loss of hypocretin-producing neurons due to a mechanism of neural destruction that indicates an autoimmune pathogenesis. There is also a strong association with HLA genes, as the 82% of patients display the DQB1*06:02 allele. The purpose of this study is to analyse the role of the HLA genes in NT1 patients and the susceptibility to autoimmune diseases. **Material and methods.** A total of 62 DNA samples from NT1 patients were analysed. Each DNA was purified through MagNApure automatic technology (Roche®, Switzerland). The DNA quantification was performed using Nanodrop spectrophotometer (Thermo Fisher Scientific®, USA) and HLA match study was done via Luminex technology (Diagnostica LongWood®, Spain). **Results.** 54 NT1 patients out of 62 (82% in NT1 vs. 22% in controls, $P<10^{-5}$) had the DQB1*06:02 allele. Moreover, 22 NT1 patients out of 62 (35.48%) had one or more autoimmunity diseases (AD) associated. Within this group, 18 displayed the DQB1*06:02 allele (81.81% in NT1 with AD vs. 22.62% in controls, $P<10^{-5}$), whereas 4 patients were DQ2 positive because the DR3 allele was significantly more frequent than in controls (75% in NT1 with AD vs. 22% in controls, P<0.001). **Conclusion.** The DQB1*06:02 allele plays an important role in NT1 patients. Furthermore, there is a high frequency of ADs in NT1, which suggests the idea that narcolepsy has an autoimmune pathogenesis.

P.A6.01.08

Celiac Disease: a dose-dependent effect of HLA-DQB.1*02 gene to genetic susceptibility in paediatric patients

***M. Fernández-Arquero**, L. N. Campo Blázquez, B. González Fernández, A. Bodas, A. García-Ron, M. T. de Santos Moreno, K. Guevara, S. Sánchez-Ramón;*
San Carlos University Hospital, Madrid, Spain.

Introduction: Celiac disease (CD) is a T cell-mediated, tissue-specific autoimmune disease which is found in genetically susceptible individuals who carry the HLA-DQB1*02 or DQB1*08 genes. This pathology causes damage to the small intestinal mucosa when gluten or related prolamines, are ingested. In these patients, the small bowel biopsies have been widely accepted as a gold standard for diagnosis. However, is barely known the effect that HLA-DQB1 gene dose has in predisposition to CD. The aim of this study is to analyse the role of the HLA-DQB1*02 genes in paediatric patients. **Material and methods**: 200 DNA samples were analysed from CD diagnosed patients (100) and healthy donors as controls (100). Each DNA was obtained with Magnapure automatic technology (Roche®, Switzerland). The DNA quantification was done through Nanodrop spectrophotometer (Thermo Fisher Scientific®, USA) and HLA match study was performed using Luminex technology (Diagnostica Longwood®, Spain). **Results**: We observed 92% of the patients displayed the DQA.1*05 and DQB.1*02 alleles, in contrast to controls: 25,6% (p<0.0001). The DR7, DQ2 and/or DR4, DQ8 haplotypes, were present in those CD patients that didn't carry the DQαβ heterodimer. Moreover, a 58% of patients displayed the DRB.1*07 allele carrying DQ2 haplotype, in contrast to controls: 3,3% (p<0.0001). **Conclusions:** We remark the role of DQB.1*02 as a strong marker of genetic predisposition in paediatric patients, which could be used as possible prognostic factor of CD. The presence of DRB.1*07, agree with an additive effect of DQ2 haplotype to that conferred by the DQ2.5 when these are present.

P.A6.01.09

Heterozygous mutation in BCL10 in a patient with T lymphopenia and ulcerative colitis

***S. García Gómez**[1,2], L. Trotta[3], R. Martinez-Barricarte[4], Y. Itan[4,5], T. Martelius[6], B. Boisson[4], S. Sanchez Ramón[7], E. Lopez-Collazo[8], M. Martín Arranz[9], E. Martín Arranz[9], L. García-Ramírez[9], J. Saarela[10], J. Casanova[11,12,13], M. Seppänen[14,15], R. Pérez de Diego[1,2];*
[1]Laboratory of Immunogenetics of Diseases, IdiPAZ, Institute for Health Research, Madrid, Spain, [2]Innate Immunity Group, IdiPaz Institute for Health Research, Madrid, Spain, [3]Institute for Molecular Medicine Finland, Helsinki, Finland, [4]St.Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, The Rockefeller University, New York, United States, [5]The Charles Bronfman Institute for Personalized Medicine, Icahn School of Medicine at Mount Sinai, New York, United States, [6]Adult Immunodeficiency Unit, Inflammation Center Helsinki University and Helsinki University Hospital, Helsinki, Finland, [7]Clinical Immunology Department, San Carlos Clinical Hospital, Madrid, Spain, [8]Innate Immunity Group, Idipaz Institute for Health Research, La Paz Hospital, Madrid, Spain, [9]Gastroenterology Department, La paz Hospital, Madrid, Spain, [10]Institute for Molecular Medicina Finland, University of Helsinki, Helsinki, Finland, [11]Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland, [12]Howard Hughes Medical Institute, New York, United States, [13]Laboratory Of Human Genetics of Infectious Diseases Necker Branch, Imagine Institute, Necker Hospital for Sick Children, Paris, France, [14]Adult Immunodeficiency Unit, Inflammation Center, Helsinki University and Helsinki University Hospital, Helsinki, Finland, [15]Rare Dusease Cebterm Children´s Hospital Helsinki University and Helsinki University Hospital, Helsinki, Finland.

Caspase recruitment domain-containing (CARD) family adaptors form heterotrimers with B-cell lymphoma 10 (BCL10) and mucosa-associated lymphoid tissue lymphoma-translocation gene 1 (MALT1). CARD-BCL10-MALT1 (CBM) complex activates nuclear factor (NF-κB) in both the innate and adaptive arms of immunity. Inherited defect of human BCL10 complete deficiency was recently reported in a child with a broad combined immunodeficiency. This patient suffered a chronic non-specific colitis with moderated lymphocytic infiltration in lamina propia. We report here a new mutation of BCL10. The patient carrying the mutation is an adult with chronic primary T lymphopenia, low B cell levels and ulcerative colitis. A heterozygous mutation in BCL10 was found (L8P). In spite of normal BCL10 expression, this patient has an impaired NF-κB-mediated function in fibroblast similar to the one observed in the first patient reported. Our results show that BCL10 L8P heterozygous mutation can be responsible of this new phenotype.

P.A6.01.10

A rare cause of immunodeficiency: glycogen storage disease type 1b

*B. Erdur, I. Parlak, M. Köse, E. Şahin, E. Özbek, **F. Genel**;*
Department of Pediatrics Dr.Behcet Uz Children's Hospital, Izmir, Turkey.

Glycogen storage diseases (GSD) are inherited metabolic diseases characterized by accumulation of glycogen in tissues. GSD type 1b effects lipid, carbonhydrate and purin metabolism and neutropenia may occur. The patients are susceptible to recurrent bacterial infections and have recurrent oral ulcers due to immune dysfunction caused by a combination of neutropenia and impaired phagocytic functions. Here we present an infant with GSD type 1b admitted for failure to thrive, abdominal distention, and developed hypoglycemia during the follow up. We want to take attention to this rare disease. A 2,5 months old girl infant admitted to our hospital for failure to thrive, abominal distention and recurrent bacterial infections. In her previous history despite appropriate brestfeeding she had inadequate weight gain. Her parents were second degree relatives. On physical examination rounded "doll's face", abdominal distention and hepatomegaly were found. Laboratory tests revealed neutropenia, elevated liver transaminases, serum lactat and uric levels and hyperlipidemia. Hypoglycemia occured during the follow up. With these clinical findings GSD type 1b was considered and genetic analysis was performed. Homozygous mutation in SLC37A4 gene was found. Enzymatic analysis supported the diagnosis. Nutritional management was regulated to prevent the child from hypoglycemia and GM-CSF was given. In infants with hepatomegaly, hypoglycemia, elevated transaminases, neutropenia and recurrent bacterial infections GSD type 1b should be considered for diagnosis. With early nutritional interventions and intensive treatment of the infections in neutropenic patients and short-term treatment with GM-CSF for serious infections, the life quality and life expectancy of the patients may be improved.

P.A6.01.11

Innate immune responses in MHC class I deficiency resulting from β2-microglobulin gene mutation

B. Kayaoglu[1], *N. Sürücü*[1], *A. Eden*[2], *H. Uçkun*[2], *M. Özışık*[2], *I. C. Ayanoglu*[1], *B. Geçkin*[1], *A. M. Acar*[1], *Ö. Ardeniz*[2], *M. Gürsel*[1];
[1]*Department of Biological Sciences, Middle East Technical University, Ankara, Turkey,* [2]*Medicine Division of Allergy and Clinical Immunology, Ege University Medical Faculty, Izmir, Turkey.*

Introduction: A novel mutation in β2-microglobulin gene has been recently identified in two Turkish siblings, resulting in low numbers of $CD8^+$ T cells and absence of MHC class I expression. Although such a phenotype would be expected to result in susceptibility to mainly viral infections, the 26-year-old male patient (one of the siblings) was fairly asymptomatic compared to his sister, who suffered from multiple forms of immune dysregulation. Herein, we aimed to investigate the underlying cause of innate immune system hyperactivation by means of functional assays.
Methods: PBMCs from patient and healthy donors were stimulated with various pattern recognition receptor (PRR) ligands and immune responses were analyzed using cytokine ELISA. STAT phosphorylation and cytosolic ROS production were assessed by flow cytometry. Gene expression was analyzed by Nanostring inflammation panel.
Results: Stimulation of cells with various PRR ligands resulted in diminished pro-inflammatory cytokine production in the patient as opposed to an increase in type I IFN secretion when compared to healthy controls. Patient PBMC spontaneously secreted IP10 in the absence of stimulation. Nanostirng gene expression analysis was consistent with protein level analysis, indicative of enhanced type I IFN signature. In addition, the patient's neutrophils were observed to produce spontaneous ROS and this patient was found to have high percentage of low density neutrophils (LDG) that was absent in healthy individuals.
Conclusion: Presence of hyperactive neutrophils and LDGs may explain resistance to bacteria while an increased type I IFN signature may explain the resistance to viral infection in this patient.

P.A6.01.12

The NGS approach in evaluation of known and novel mutations associated with X-linked agammaglobulinemia (XLA)

I. Kofiadi[1], *I. Manto*[1], *E. Latysheva*[1], *T. Latysheva*[1], *A. Nikiforova*[2], *G. Gudima*[1], *M. Khaitov*[1];
[1]*FSBI "NRC Institute of Immunology" FMBA of Russia, Moscow, Russian Federation,* [2]*DNA-Technology, JSC, Moscow, Russian Federation.*

The XLA specified as primary humoral immunodeficiency with apparent genetic component. The disease predominantly manifested in patients bearing mutations in BTK gene and characterized by the failure to produce mature B-lymphocytes. The differential diagnosis from other PIDs, characterized by similar symptoms, but requiring other treatment, is quite challenging without genetic confirmation. Currently the most effective and relevant approach for target genetic screening is NGS. We developed the test system covering 19 exons of BTK gene suitable for Ion PGM platform. The study enrolled 7 patients with possible XLA (ESID criteria) between 18 and 36. 4 patients showed single pathogenic mutations. One patient had 3 different mutations and another one had 4 different mutations. In addition, one patient with apparent symptoms of XLA had no mutations in BTK gene. The results were compared with most relevant and complete databases: BTKbase and LOVD, as well as checked for citation in PubMed. Analysis showed that 5 mutations were not indexed in databases. Two of them NM_000061.2:c.241-1G>A and NM_000061.2:c.1178-1G>A were previously described by Stewart D.M. (2001) and Toth B. (2009) respectively. Three mutations considered to be new and, presumably, pathogenic: two deletions -NP_000052.1:p.Met570del/NM_000061.2:c.1708_1710delATG; p.Cys145Alafs*31/NM_000061.2:c.433delT and one SNP - NM_000061.2:c.1909-1G>A. The obtained results confirm the utility of NGS approach in evaluation of BTK mutation profile. The genetic data will be used in further follow-up and characterization of T-cell immunity of XLA patients. Non-confirmed diagnosis will be reconsidered and subjected for further investigation. New mutations will be submitted to BTK mutation databases.

P.A6.01.13

Characterization of the monocyte/macrophage compartment in a patient with a novel *CSF1R* mutation causing hereditary diffuse leukoencephalopathy with spheroids

D. Quandt[1], *T. Kraya*[2], *T. Pfirrmann*[3], *A. Kindermann*[1], *J. Kohlhase*[4], *D. Stoevesandt*[5], *K. Hoffmann*[6], *P. Villavicencio-Lorini*[6];
[1]*Departement of Anatomy and Cell Biology, University of, Halle, Germany,* [2]*Department of Neurology, University of, Halle, Germany,* [3]*Institute of Physiological Chemistry, University of, Halle, Germany,* [4]*SYNLAB MVZ Humangenetik Freiburg, Freiburg, Germany,* [5]*Department of Radiology, University of, Halle, Germany,* [6]*Institute of Human Genetics, University of, Halle, Germany.*

Colony-stimulating factor 1 receptor (CSF1R) is a tyrosine kinase transmembrane protein that mediates proliferation, differentiation and survival of monocytes/macrophages and microglia by activation through the cytokine CSF1 or IL-34. Interestingly, *CSF1R* gene mutations cause hereditary diffuse leukoencephalopathy with spheroids (HDLS), an autosomal-dominantly inherited microgliopathy leading to rapid neurocognitive decline with high lethality. By detailed clinical assessment and targeted gene sequencing we identified a novel *CSF1R*-indel mutation in a 44-year old female patient with a complex neuropsychiatric clinical presentation, initial signs of cerebral gliosis, and positive family history. By FACS analysis of peripheral blood monocytes we detected marginal elevated cell surface protein levels of the CSF1 (CD115) receptor. Interesting, we found an increased number of total blood monocytes. Subdividing monocyte populations by flow cytometry revealed a decreased frequency of non-classical monocytes in the blood of the patient. Of particular note, we discovered an increased Tyr723 autophosphorylation by intracellular flow cytometry in peripheral monocytes, indicating a gain-of-function effect of the mutation. Ongoing analyses will reveal whether there is an altered potential of macrophage differentiation and polarization. Furthermore pharmacological inhibition of the CSF1R by the use of GW2580, shown to arrest microglial proliferation, will be applied in vitro to study potential treatment options for this novel *CSF1R*-indel mutation.

P.A6.01.14

Investigation of heterozygous *NFKB1* variants in a common variable immunodeficiency cohort

C. Schröder[1], *T. Witte*[1], *R. Jacobs*[1], *T. Dörk*[1], *B. Grimmbacher*[2], *R. E. Schmidt*[1], *F. Atschekzei*[1];
[1]*Hanover Medical School, Hannover, Germany,* [2]*Center for Chronic Immunodeficiency, Freiburg, Germany.*

The transcription factor nuclear factor kappa B (NF-κB1) is sequestered within the cytoplasm of every cell. During activation of the canonical NF-κB pathway, NF-κB1 translocates to the nucleus and binds to the promotors of its target genes. NF-κB1 has been linked with a diversity of diseases, including asthma, AIDS, diabetes and cancer. More recently, it has been shown that *NFKB1* mutations could lead to haploinsufficiency of the active subunit p50 and therefore could cause the CVID phenotype. CVID as a syndrome comprises a heterogeneous group of molecular diseases, characterized by a significant hypogammaglobulinemia of unknown cause.
Genomic DNA for targeted-NGS was isolated from whole blood. Detected mutations were validated by Sanger sequencing. PBMCs were isolated by density gradient centrifugation and stimulated with PMA plus ionomycin and analyzed using immunoblotting with antibodies against NF-κB1 p105 and p50.
In our study we identified five novel heterozygous mutations in *NFKB1* in seven patients by targeted NGS. Among those, one frameshift deletion, three single base-pair insertions, one missense and one splice site mutation were observed. *NFKB1* mutations occur in our CVID cohort with a prevalence of 1:30. In all affected members of three families, their mutations lead to a reduction of the active NF-κB1 subunit p50. Nevertheless, the mutations segregate with incomplete penetrance in families.
Mutations in *NFKB1* lead to reduced levels of p50. Due to the incomplete segregation of penetrance, other causes, like epigenetic changes or intestinal microbiome alteration may promote the onset of disease.
Supported by DZIF TTU 07.801

P.A6.01.15

Molecular and bioinformatics characterization of a novel mutation in *STXBP2* gene: a case report on Familial Hemophagocytic Lymphohistiocytosis

L. Viñas-Gimenez[1,2], *L. Donadeu*[1,2], *E. Alvarez de la Campa*[1,2], *R. Colobran*[1], *A. Català*[3], *X. de la Cruz*[2], *L. Alsina*[3], *J. Sayos*[1,2], *M. Martínez-Gallo*[1];
[1]*Vall d'Hebron University Hospital, Barcelona, Spain,* [2]*Institut de Recerca Vall d'Hebron (VHIR), Barcelona, Spain,* [3]*Hematology Department, Hospital Sant Joan de Déu., Barcelona, Spain.*

Familial Hemophagocytic Lymphohistiocytosis (FHL) is a rare autosomal recessive disorder characterized by uncontrolled immune activation but ineffective response. Disease-causing mutations have been reported in several genes: *PRF1*-FHL2, *UNC13D*-FHL3, *STX11*-FHL4 and STXBP2-FHL5. All FLH forms are first diagnosed based on clinical symptoms and laboratory findings following accepted guidelines. We present a 2-year-old baby-boy from non-consanguineous parents that developed two episodes of EBV-HLH in 4 months. His older brother died at the age of 3 years of HLH also triggered by EBV infection. Functional test revealed impaired cytotoxicity, reduced CD107a Mean-Fluorescence-Intensity (MFI) but normal percentage of NK cells expressing CD107). Genetic analysis identified a compound heterozygous mutations in *STXBP2* gene, one a previously reported in exon 15 (c.1247-1G>C) and the second a novel mutation in exon 9 (c.728T>G (p.L243R)). The L243 residue is evolutionarily highly preserved and PyMOL prediction indicates that sits at the centre of a rich network of interactions important for stabilizing domains 2 and 3 of the STXBP2 protein. Transfection of constructs with the mutated and WT sequences into COS7 cells resulted in good level of WT but no expression of STXBP2-L243R, confirming PyMOL prediction. Overall the known effect of the allele pV417LX126 plus the in-silico analysis and transfection experiments of the L243R allele indicate that all the remaining degranulation function of STXBP2 is attributable to the pV417Lfs allele. This would explain the results of functional assays, i.e., impaired cytotoxicity with reduced CD107a surface expression as measured by MFI but normal as percentage of CD107a+ NK cells after stimulation.

P.A6.01.16

Clinical and Immunologic Data in Two Groups of Familial and Sporadic Patients with Common Variable Immunodeficiency

***R. Yazdani**, A. Valizadeh, G. Azizi, H. Abolhassani, A. Aghamohammadi;*
Research Center for Immunodeficiencies (RCID), Tehran, Iran, Islamic Republic of.

Introduction: Common variable immunodeficiency (CVID) is the most frequent symptomatic primary immunodeficiency disease and its prevalence varies significantly among different population. Minority of CVID patients present a familial aggregation suggesting a higher probability of heritable genetic defects. **Methods:** A total of 235 registered CVID patients were evaluated in this cohort study. Familial and sporadic patients were stratified and demographic information, clinical records, laboratory and molecular data were compared among these two groups of patients. **Results:** Multiple cases were identified in 12 families (30 patients) and sporadic presentation in 120 cases. The rate of parental consanguinity (83.3%) and clinical presentation of lymphoid malignancy (20.7%) were predominant in familial CVID patients, whereas significantly increased recurrent upper respiratory infections were recorded in sporadic patients (0.3 infections per year). Probands of familial group were presented with a higher severity score resulting in a profound mortality rate (41.7% after 30-years follow-up) comparing to the non-proband CVID patients in the same families with a lowered diagnostic delay. **Conclusion:** Familial CVID patients had a specific signature in clinical presentation and immunologic profile and a high consanguinity in this group of patients suggests a Mendelian trait with an autosomal recessive inheritance pattern. Diagnosis of an index patient within a multiple case families significantly improves the diagnostic process and outcomes of the yet asymptomatic patients

P.A6.01.17

First case with cernunnos deficiency from the national Iranian registry

***R. Yazdani**[1], H. Abolhassani[1], J. Tafaroji[1], G. Azizi[1], A. Hamidieh[1], J. Chou[2], R. S. Geha[2], A. Aghamohammadi[1];*
[1]Research Center for Immunodeficiencies (RCID), Tehran, Iran, Islamic Republic of, [2]Division of Immunology Boston Children's Hospital, and Department of Pediatrics, Harvard Medical School, Boston, USA, Boston, United States.

Introduction: Severe combined immunodeficiency (SCID) is a heterogeneous group of genetic disorders. Cernunnos is a DNA repair factor that is involved in Non-homologous end-joining (NHEJ) process. Impairment in Cernunnos leads to a genetic disease characterized by neural disorders, immunodeficiency and increased radiosensitivity.
Methods: We obtained clinical manifestations and immunological findings by reviewing hospital records. Mutation analysis was done by whole exome sequensing and the mutation was confirmed by sanger sequencing.
Results: We herein describe a SCID patient with T- B+ phenotype who had a mutation in Cernunnos gene and manifested recurrent infections, microcephaly and growth retardation with hypogammaglobulinemia. Furthermore, our patient was associated with BCG adenitis and autoimmunity that less is observed in patients with Cernunnos deficiency.
Conclusions: In contrast to previous reported Cernunnos-deficient patients, our patient had normal B-cell number along with normal IgA and IgM, suggesting a leaky form of Cernunnos deficiency due to residual count of B cells in our patient. Cernunnos deficiency should be considered in children with recurrent bacterial infections, microcephaly and growth retardation, in spite of having normal B-cell as well as normal IgM and IgA level.

P.A6.02 Lessons learned from the genetic defects - Part 2

P.A6.02.01

Association of interferon regulatory factor (IRF 5) gene (rs2280714) SNP with systemic lupus erythematosus patients

*H. Hamid[1], A. Waqar[1], S. Ullah[1], A. Jamal[1], S. Pervaiz[2], S. Jahan[3], N. Afzal[3], **B. Adil**[4];*
[1]ICBS, Faculty of Health and Allied Sciences, Lahore, Pakistan, [2]King Edward Medical University, Lahore, Pakistan, [3]University of Health Sciences, Lahore, Pakistan, [4]Faculty of Health and Allied Sciences, Lahore, Pakistan.

Background- Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disorder. Center for disease control (CDC)-Atlanta estimated 1.8 to 7.6 per 100,000 people affected with SLE per year in the USA. SLE is a heterogeneous disease. Various genome wide association studies have shown association of interferon regulatory factor 5 (IRF5) gene with SLE. Therefore, this study was aimed and designed to determine single nucleotide polymorphism (SNP) in IRF5 gene restriction site (rs2280714) in local SLE patients and healthy controls.
Objective- To determine the frequency IRF5 (rs2280714) gene polymorphism in SLE patients and healthy controls
Materials and methods- It was a case control study. Eighty samples were recruited for each of the two study groups. DNA extraction was carried out using standard phenol-chloroform technique. Further, samples were processed by PCR-RFLP (Restriction fragment length polymorphism) conventional method. Polymorphism analysis and allele frequencies were compared between groups using chi-square test.
Results- It revealed that SNP in IRF5 gene (rs2280714) is not associated with SLE in Pakistani population. CC genotype is more frequent among various major clinical manifestations of SLE.
Conclusion- This study might incorporate with even better clarification of underlying etiological and prognostic factors regarding SLE.

P.A6.02.02

Identification of *Tyk2* loss-of-function mutations in a cohort of B cell acute lymphoblastic leukemia patients and characterization of B cell dysregulated function in *TYK2*-deficient mice

***I. Bodega-Mayor**[1], E. A. Turrubiartes[1], I. Cortegano[2], M. L. Gaspar[2], B. de Andrés[2], E. Fernández-Ruiz[1];*
[1]Instituto de Investigación Sanitaria Princesa (IIS-IP), Madrid, Spain, [2]Centro Nacional de Microbiología, Instituto de Salud Carlos III (ISCIII), Madrid, Spain.

B-acute lymphoblastic leukemia (B-ALL) is the most prevalent childhood hematological malignancy. In these patients, mutations in genes associated with JAK/STAT signaling are frequent. Tyrosin kinase 2 (TYK2) is a member of the Janus kinase family (JAKs), involved in several cytokine signaling pathways, and therefore it is important in hematopoiesis and immune system. *TYK2*-null mice show deficient tumour surveillance and resistance to LPS-induced septic shock, however the implication of B cells in these phenotypes is still unknown.
Using next-generation sequencing, we have sequenced DNA from 65 B-ALL patients at diagnosis and identified two mutations not previously reported and eight polymorphisms in *Tyk2*. We have tested in vitro these new mutations and one polymorphism, and they showed an impaired signaling in response to IFN-alpha, resembling the kinase-dead form of the protein.
Additionally, we have studied the B cell compartment of *TYK2*-null mice after in vivo immunization. We have observed an altered humoral B cell response to a T-dependent antigen (NP-OVA), with a significant reduction of memory switched B cells and specific IgM and IgG2a sera. These mice respond also deficiently after the T-independent stimulation with LPS, by decreasing the total number of marginal zone B cells and increasing follicular B cells.
In conclusion, 3% of ALL-B patients studied carried new loss-of-function mutations in *Tyk2*. In mice, TYK2 is necessary for an appropriate response to T-dependent and T-independent antigens and the homeostasis of the B cell compartment. These data postulate TYK2 as a key player in the regulation of the B cell function.

P.A6.02.03

Functional characterization of disease associated variants of human complement Factor H-related protein 5

***M. Cserhalmi**[1], B. Uzonyi[1], D. Csuka[2], K. Uray[3], A. Iliás[1], Z. Prohászka[2], M. Józsi[1];*
[1]Department of Immunology, Eötvös Loránd University, Budapest, Hungary, [2]3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary, [3]MTA-ELTE Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös Loránd University, Budapest, Hungary.

Complement is a major humoral arm of innate immunity that plays important roles in the protection against infections, regulation of inflammation and disposal of immune complexes and cellular waste. Dysregulation of the complement alternative pathway is involved in the pathogenesis of several diseases, including the kidney disease C3 glomerulopathy. Factor H is a main inhibitor of the alternative pathway; however, the role of the factor H-related FHR proteins is less characterized. FHR5, which consists of 9 complement control protein (CCP) domains, was described to bind C-reactive protein (CRP) and C3b. Our aim was to map the ligand binding sites in FHR5 and characterize FHR5 variants described in patients. Wild type and mutant FHR5 were expressed in insect cells. Binding studies were performed by ELISA and surface plasmon resonance (SPR). Fifteen amino acid-long peptides of FHR5 CCPs 3-9 and mutant peptides were synthesized on Mimotopes NCP gears. SPR experiments showed that FHR5 G278S and R356H association (k_a) to C3b was decreased, but FHR5 R356H dissociation (k_d) from C3b was slower, while that of G278S was faster than the wild type FHR5. Similar results were obtained by ELISA, indicating weaker C3b binding by FHR5 G278S. Epitope mapping revealed that C3b binding to FHR5 peptides with K144N, V170M, N178S was increased and binding of CRP to the R356H and M514R mutant peptides was decreased compared to wild type peptides. Altogether, our results identify amino acids within FHR5 involved in binding C3b and CRP, and reveal altered ligand binding by some mutant FHR5 proteins.

P.A6.02.04
NFATc1 activity is indispensable for T cell development

S. Giampaolo[1], *S. Klein-Hessling*[1], *F. Berberich-Siebelt*[1], *E. Serfling*[1], *A. Patra*[2];
[1]*Institute of Pathology and Comprehensive Cancer Center Mainfranken, University of Würzburg, Würzburg, Germany,* [2]*Institute of Translational and Stratified Medicine, Peninsula Schools of Medicine and Dentistry, University of Plymouth, Plymouth, United Kingdom.*

In lymphoid cells NFATc1, NFATc2 and NFATc3 transcription factors are expressed and involved in antigen-receptor signaling. Specifically, NFATc1 plays a critical role in thymocyte differentiation and survival. During T cell development in the thymus, the $CD4^-CD8^-$ double-negative (DN) cells differentiate to the $CD4^+CD8^+$ double-positive (DP) stage where they undergo the process of positive- and negative- selection to finally give rise to the $CD4^+$ or $CD8^+$ single positive (SP) T cells. Based on the expression of CD25 and CD44 molecules, the DN thymocytes again consist of four distinct populations: $CD44^+CD25^-$DN1, $CD44^+CD25^+$DN2, $CD44^-CD25^+$DN3 and the $CD44^-CD25^-$DN4 cells. The DN1-DN3 cells are critically dependent on IL-7 signaling for their survival and differentiation. We have shown previously that IL-7 signaling activates NFATc1 in the preTCR-negative DN thymocytes in a Jak3-dpendent manner leading to its nuclear translocation. Survival of DN thymocytes is partly due to an NFATc1-mediated transcriptional upregulation of the pro-survival molecule Bcl2 in these cells. *In vivo* binding studies by ChIP-Seq analysis of thymocytes confirmed the binding of NFATc1 to the *Bcl2* gene locus. The indispensability of NFATc1 activity was evident as a hematopoietic cells-specific ablation of NFATc1 activity resulted in an arrest of thymocyte differentiation at the DN1 stage leading to lymphopenia. On the other hand, overexpression of a constitutively active NFATc1 resulted in an impaired transition of DN3 cells to the DN4 stage, again leading to lymphopenia. These observations suggest that a threshold level of NFATc1 activity is critical for efficient T cell development.
José Carreras Leukämie- Stiftung

P.A6.02.06
Chronic mucocutaneous candidiasis associated with a rare molecular defect: TRAF3IP2 mutation

N. E. Karaca, *A. Aykut, E. Parıltay, A. Durmaz, O. Cogulu, G. Aksu, N. Kutukculer;*
Ege University Medical School, Izmir, Turkey.

Chronic mucocutaneous candidiasis (CMC) is characterized by recurrent or persistent infections of skin, mucous membranes or nails with *Candida albicans* and sometimes staphylococcal infections. Patients with autosomal dominant (AD) Hyper-IgE syndrome (STAT3 deficiency) and STAT1 gain-of-function mutation, AR deficiencies in IL-12RB1, IL-12p40, CARD9 or APACED (autoimmune-poliendocrinopathy-candidiasis-ectodermal dystrophy) syndrome develop CMC as a major infectious phenotype. IL-17 receptor A/C F and ACT1 defects in the IL-17 signaling pathway also lead to CMC.
A 19-month-old-girl, born to second-degree consanguineous parents, referred with recurrent oral trush and skin eruptions. Elder brother had also CMC. Physical examination revealed oral mucosal candidiasis and multiple pustular eruptions all over the body. Hypergammaglobulinemia was observed. Absolute neutrophil counts, lymphocyte subgroups, IgE level and oxidative burst activity were normal. *Candida albicans* grew in the mouth swab culture and *S. Aureus* was isolated from the wound swab. A homozygous c.1569G> C (p.Trp532Cys) mutation was detected in the *TRAF3IP2* gene with the targeted next generation sequencing-based Ion AmpliSeq ™ Primer Immunodeficiency Panel. The parents were shown to carry the same mutation as heterozygous. All complaints were recovered by the treatment with trimetoprim-sulfometaxazole and fluconazole prophylaxis.
TRAF3IP2 (TNF-Receptor-Associated-Factor3-Interacting-Protein2) encodes the Act1 molecule, an adapter protein with ubiquitin-ligase activity that binds the IL-17receptor to downstream signaling pathways. ACT1 defects affecting the IL-17signaling pathway in AR CMC cases should also be kept in mind in the differential diagnosis. To our knowledge, this is the first ACT1 deficiency patient after two siblings who were published in 2013 by Boisson B et al.

P.A6.02.07
Overproduction of XBP1s protects from ER stress induced apoptosis in cystic fibrosis primary monocytes

S. Lara Reyna[1], *T. Scambler*[1], *J. Holbrook*[1], *C. Wong*[1], *H. Jarosz-Griffiths*[1], *F. Martinon*[2], *S. Savic*[1], *D. Peckham*[1], *M. F. McDermott*[1];
[1]*Univeristy of Leeds, Leeds, United Kingdom,* [2]*Univeristy of Lausanne, Lausanne, Switzerland.*

The cystic fibrosis transmembrane regulator (CFTR) is a transmembrane protein, involved in the transport of bicarbonate and chloride ions. Mutation of the CFTR causes cystic fibrosis (CF), resulting in recurrent pulmonary infections and autoinflammation mainly in the lungs. The CFTR is assembled and modified in the endoplasmic reticulum (ER) and, when mutated, accumulation of the CFTR leads to activation of the unfolded protein response (UPR). The UPR comprises three ER transmembrane proteins, known as PERK, IRE1, and ATF6. Chronic UPR activation, through its PERK arm, is linked with apoptosis through accumulation of CHOP. PERK activation can be inhibited by $p58^{IPK}$ production, which is induced by IRE1 activation through XBP1s. The aim of this study was to investigate UPR activation in CF patients.
Patients' peripheral blood mononuclear cells (PBMCs), primary monocytes, and human bronchial epithelial cells (HBEC), were used to evaluate UPR activation, using qPCR and flow-cytometry. LPS, tunicamycin and thapsigargin were used as cellular UPR stimulants to assess UPR activation.
Gene expression revealed a significant increase in *XBP1s* in HBEC lines, PBMCs, and monocytes from CF patients. IRE1α protein expression was also increased in the three CF cell lines. Furthermore, CF monocytes pre-treated with the IRE1 inhibitor, 4μ8C, showed a significant increase in the activity of PERK, including ATF4, GADD34, and CHOP, after UPR activation. Finally, PERK overactivation correlated with the downregulation of $p58^{IPK}$ in CF monocytes after 4μ8C pre-treatment.
Data suggest that misfolded CFTR proteins induce ER stress in CF. Furthermore, XBP1s overproduction protects CF cells from CHOP-induced apoptosis.

P.A6.02.08
Linker for activation of T cells (LAT) inhibits development of aggressive thymic lymphomas by downregulating Notch-1 and pTa expression

K. Marek-Bukowiec[1,2], *M. Zalas*[1], *M. Lisowska*[1], *B. Malissen*[3], ***A. Miążek***[1,4];
[1]*Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland,* [2]*Ośrodek Badawczo Rozwojowy, Wojewódzki Szpital Specjalistyczny, Wroclaw, Poland,* [3]*Centre d"immunologie de Marseille Luminy, Marseille, France,* [4]*Wroclaw University of Envronmental and Life Sciences, Wroclaw, Poland.*

Linker for Activation of T Cells (LAT) is a raft-associated, transmembrane adaptor protein whose expression promotes expansion and selection of thymic T cell precursors. In LAT deficient mice (*LAT-/-*) thymocyte development is arrested at the CD4-8-25+ (DN3) stage but this block can be relieved when these mice are crossed with a transgenic line bearing a chronically active allele of LCK kinase (LCK^{Y505F}). Similar effect can also be observed in CD3e-/-LCK^{Y505} crosses accept that the former but not the latter mice develop fully penetrant, aggressive thymic lymphoma (T-ALL) characterized by a CD4lowCD8+ (DP) phenotype. Here, we aimed at identifying gene expression differences underlying the LCK driven DN3 to DP transition in *LAT-/-*LCK^{Y505F} versus control CD3e-/-LCK^{Y505} mice. We focused on gene transcripts *representative* of 15 different *signaling pathways* related to T-ALL. Comparative, transcriptomic analysis of pre-leukemic DP thymocytes from 3 week old mice led to identification of hyperactive Notch signaling pathway as an early, critical, LCK-driven oncogenic signal leading to the initiation of T-ALL. Our result imply that the functional LAT signalosome downregulates Notch and its target genes (i.e. pTa) at the DP stage of thymocyte development thus contributing to the restoration of their sensitivity to negative selection preventing oncogenic transformation.

P.A6.02.09
Celiac disease in patients from Cantabria (northern Spain): distribution of risk HLA haplotyes

L. Riesco-Davila, F. Ausin Ortega, ***M. Lopez-Hoyos****, J. Ocejo-Vinyals;*
Hospital Universitario Marqués de Valdecilla, Santander, Spain.

Celiac disease is one of the most prevalent genetically determined autoimmune diseases, and one of the diseases with strongest association with particular HLA haplotypes, specifically with DQ2 and DQ8.
Regarding the risk of the HLA haplotypes, several discrepancies among populations have been found, mainly due to no take into account the difference between the HLA DQ2.2 and DQ2.5 molecules.
For this reason, we genotyped 781 celiac disease patients (492 children < 15 years old, and 289 adults) who fulfilled the ESPGHAN criteria for this disease by using PCR-SSO in a Luminex platform.
In our population, when we analyzed together children and adults, we found some differences in the frequency of the haplotypes associated with an increased susceptibility to celiac disease compared with previously reported populations, and in the risk that these haplotypes conferred to the disease, mainly in the distribution of DQ2.2/DQ2.2, DQ2.2/x, DQ2.2/DQ7 and DQ8 either in homocigosity or heterocigosity. Interestingly, half of DQ2.2/x carried the DQA1*04 in trans position. We think that the main reasons of these differences are: a) the different prevalence of these haplotypes among populations and b) lack of separate analysis between DQ2.2. and DQ2.5.

P.A6.02.10

Association of HLA alleles and haplotypes with susceptibility to hidradenitis supurativa

*G. Ocejo-Vinyals, M. González-Gay, **L. Riesco-Davila**, I. Vilanova, J. Cantos Mansilla, C. Duran, R. Blanco, M. Gonzalez-Lopez;*
Hospital Universitario Marqués de Valdecilla, Santander, Spain.

Hidradenitis suppurativa (HS) is a chronic, inflammatory skin disease of the hair follicle characterized by relapsing painful inflammatory nodules, abscesses and fistula tracts in the apocrine gland-bearing areas of the body. Its pathogenesis is not completely understood. Familial HS have shown autosomal-dominant inheritance caused by mutations in the γ-secretase genes (*PSENEN*) but most of the HS patients do not present these mutations. To date, only one published study tried to study the role of HLA A, B or DR antigens as a factor contributing to a genetic susceptibility to HS without significant results. 106 patients with HS and 262 healthy controls were studied for the distribution of the HLA-A, B, C, DRB1, DQA1 and DQB1 alleles by using a PCR-SSOP technique and Luminex analysis. We found differences in the distribution of HLA, A, B, DRB1, DQA1 and DQB1 alleles. After Bonferroni correction, only HLA class II remained significant. DRB1*07 (p=0.026, OR 0.45 IC95% 0.27-0.74) and DQB1*02 (p=0.0005, OR 0.45 IC95% 0.27-0.67) were found to be more frequent in controls than in HS patients. By contrast, DQB1*03:01 was more frequent in HS patients (p = 0.00007, OR 2.36, IC95% 1.60-3.50). Haplotype DRB1*07-DQA1*02-DQB1*02 was found to be more frequent in healthy controls (p = 0.0005, OR 0.35, IC95% 0.19-0.62). To the best of our knowledge, this is the first reported association of HLA alleles with susceptibility or protection to HS. DQB1*03:01 (DQ7) would be an allele predisposing to HS whereas DRB1*07 and DQB1*02 alleles, and DRB1*07-DQA1*02-DQB1*02 haplotype would confer protection against HS.

P.A6.02.11

Association between KIR genes and hidradenitis suppurativa in Cantabria (northern Spain)

*M. Gonzalez-Gay, J. Ocejo-Vinyals, **L. Riesco-Davila**, I. Vilanova, J. Cantos Mansilla, C. Duran, R. Blanco, M. Gonzalez-Lopez;*
Hospital Universitario Marqués de Valdecilla, Santander, Spain.

Hidradenitis suppurativa (HS) is a chronic, inflammatory skin disease of the hair follicle characterized by relapsing painful inflammatory nodules, abscesses and fistula tracts in the apocrine gland-bearing areas of the body, most commonly in the axillae, inguinal and anogenital regions. To date, no study has looked for the role of the killer cell immunoglobulin-like receptors genes (KIR) in the pathogenesis of this disease. KIR, found on the surface of natural killer (NK) cells, play a key role in controlling the innate response. To study the role of the presence / absence of KIR genes in the pathogenesis of HS. In the same way, KIR haplotype and genotype distribution is analysed.A total of 106 patients with HS and 262 age and sex-matched healthy controls were studied for the presence / absence of KIR genes by PCR-SSO and Luminex analysis. We only found a weak difference in the distribution of KIR genes between HS patients and healthy controls. Only KIR2DL3 was found to be less frequent in HS patients vs healthy controls (79 % vs 89.8%, p = 0.13 OR 0.42 IC95% 0.22-0.81) suggesting a protective role against HS. This is the first study trying to find an association of KIR genes with HS. Our results suggest that KIR genes do not influence in a significant way on resistance/susceptibility to HS.

P.A6.02.12

PTPN22 gene polymorphisms in Iranian patients with Ulcerative Colitis

***M. Sadr**[1], N. Soleimanifar[1], N. Ebrahimidaryani[2], N. Rezaei[2];*
[1]Molecular Immunology Research Center,Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of, [2]Tehran university medical of science, Tehran, Iran, Islamic Republic of.

Inflammatory bowel disease (IBD) is an idiopathic chronic recurrent gastrointestinal inflammatory disorder, which categorizes based on clinical, histological and immunological findings into two main forms of Crohn's disease (CD) and ulcerative colitis (UC). PTPN22 gene located on chromosome 1p13 and expressed exclusively in immune cells, encodes a lymphoid-specific intracellular protein tyrosine phosphatase which, is involve in T cell receptor signaling pathway. Since BD is an autoimmune disease, this study was done to examine five functional polymorphisms of PTPN22 gene to illustrate the role of this gene in this disease.
Using the Real-time PCR allelic discrimination method, 67 samples of unrelated patients with UC and 93 samples of healthy controls were genotyped. Using Fisher exact test or Chi-square test, genotypic and allelic frequencies were estimated.
The results of our study indicated a remarkable correlation of alleles and genotypes at rs1310182 as follow T allele (OR = 0.33 - 0.83, 95 % CI = 0.53, P <0.01), C allele (OR = 1.19 - 2.94, 95 % CI = 1.87, P <0.01), TT genotype (OR = 0.19 - 0.94, 95 % CI = 0.43, P= 0.03) and CC genotype (OR = 1.13 - 4.76, 95 % CI = 2.32, P <0.01) in UC.
This association study showed that rs1310182 SNP of PTPN22 could have role in UC; however, more researches on this topic are needed.

P.A6.02.13

Pathogenic *NFKB2* variant in the ankyrin repeat domain causes defective class-switched B cells and variable T cell defects *ex vivo*

***P. Tuijnenburg**[1], H. Lango Allen[2], G. J. de Bree[3], S. Savic[4], M. H. Jansen[1], S. O. Burns[5], C. Stockdale[4], I. Simeoni[2], E. M. van Leeuwen[6], J. E. Thaventiran[7], T. W. Kuijpers[1];*
[1]Department of Pediatric Hematology, Immunology and Infectious Diseases, Academic Medical Center, Amsterdam, Netherlands, [2]Department of Haematology, University of Cambridge, Cambridge, United Kingdom, [3]Department of Internal Medicine, Academic Medical Center, Amsterdam, Netherlands, [4]Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, St James's University Hospital, Leeds, United Kingdom, [5]Department of Immunology, Royal Free London NHS Foundation Trust, University College London Institute of Immunity and Transplantation, London, United Kingdom, [6]Department of Experimental Immunology, Academic Medical Center, Amsterdam, Netherlands, [7]Department of Medicine, University of Cambridge, Cambridge, United Kingdom.

Introduction: Genetic studies are identifying an increasing number of monogenic causes of Common Variable Immunodeficiency (CVID), a heterogeneous disorder characterized by recurrent infections, low serum immunoglobulins and poor vaccination responses. The cellular phenotype of those monogenic immunodeficiencies carries important information on major signaling pathways such as the canonical and non-canonical NF-κB pathway. The effect of heterozygous *NFKB1* mutations on lymphocytes have recently been characterized, however phenotype and function in heterozygous *NFKB2* mutations are not yet described in full detail. **Methods:** We studied 2 unrelated CVID pedigrees with 4 cases of pathogenic stop gain variants (c.1903C>T) in the ankyrin repeat domain (ARD) of NF-κB2, leading to a premature truncation of the protein at p.Arg635Term (R635X) identified by Whole Genome Sequencing (WGS) as part of the NIHR BioResource - Rare Disease study. We performed extensive immunophenotyping and CFSE-labeled PBMC cultures to examine B and T cell proliferation, plasmablast differentiation and immunoglobulin production. **Results:** *NFKB2* R635X cases showed reduced class-switched memory B cells but were able to induce (immature) plasmablasts, yet unable to produce IgG and IgA *ex vivo*. Moreover, *NFKB2*-defective B cells differed from patients with NF-κB1 loss-of-function by the absence of an expanded $CD21^{low}$ B cell population, associated with autoimmunity. An abnormal *ex vivo* T cell phenotype was observed in the two clinically affected index cases, which is currently being further investigated. **Conclusion:** In conclusion, pathogenic stop variants in the ARD of *NFKB2* can cause CVID with an abnormal B cell phenotype and variable T cell defects.

P.A6.02.14

B cell receptor deep sequencing shows altered somatic hypermutation patterns in patients with defects in base excision repair and mismatch repair

*H. IJspeert[1,2], **P. A. van Schouwenburg**[1], I. Pico-Knijnenburg[1], J. Loeffen[3], L. Brugieres[4], G. J. Driessen[5], C. Blattmann[6], M. Suerink[7], D. Januszkiewicz-Lewandowska[8], A. A. Azizi[9], M. G. Seidel[10], H. Jacobs[11], M. van der Burg[1,2];*
[1]Department of Immunology, Erasmus MC University Medical Center, Rotterdam, Netherlands, [2]Department of Pediatrics, Leiden University Medical Center, Leiden, Netherlands, [3]Department of Pediatric Oncology and Hematology, Sophia Children's Hospital, Rotterdam, Netherlands, [4]Department of Pediatric and Adolescent Oncology, Gustave Roussy Cancer Campus, Villejuif, France, [5]Department of Paediatrics, Juliana Children's Hospital/Haga Teaching Hospital, The Hague, Netherlands, [6]Department of Pediatric Hematology and Oncology, Palliative Care, Olgahospital Klinikum Stuttgart, Stuttgart, Germany, [7]Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands, [8]Department of Pediatric Oncology, Hematology and Transplantology, Poznan University of Medical Sciences, Poznan, Poland, [9]Department of Pediatrics and Adolescent Medicine, Medical University Vienna, Vienna, Austria, [10]Research Unit Pediatric Hematology and Immunology, Division of Pediatric Hematology-Oncology, Department of Pediatrics and Adolescent Medicine, Medical University Graz, Graz, Austria, [11]Division of Tumor Biology and Immunology, The Netherlands Cancer Institute, Amsterdam, Netherlands.

The generation of high affinity antibodies is dependent on somatic hypermutation (SHM). SHM is initiated by the activation induced cytosine deaminase (AID) which generates U:G mismatches in the B-cell receptor encoding genes. Error-prone and error-free processing of U:G mismatches creates a typical spectrum of point mutations. Limitations in obtaining genetically defined patient material has prohibited the generation of SHM data sets to test if and to what extend insights on SHM in mice apply to humans.
We performed next generation sequencing of the B-cell receptor heavy chain locus in a unique group of patients with bi-allelic mutations in genes involved in BER (*UNG*), or MMR (*MSH2, MSH6,* or *PMS2*). Our ARGalaxy analysis pipeline revealed a selective skewing of SHM patterns in all patients emphasizing the importance of UNG, MSH2, MSH6 and PMS2 in processing SHM intermediates. Our results are best compatible with a five pathway model of SHM: (1) replication opposite U generates C/G to T/A transitions; (2) translesion synthesis across UNG-generated abasic site generates C/G transitions and transversions; (3) long-patch BER involving UNG and polymerase eta generates MSH2/MSH6-independent A/T mutations; (4) a major MSH2/MSH6 dependent A/T mutation pathway involving polymerase eta; (5) the MSH2/MSH6 and UNG hybrid pathway that generates a substantial proportion of G/C transversions. PMS2 is important in the generation of SHM, however its exact contribution remains unclear.
This study is the first applying NGS on genetically well-defined human B cell samples and establishes the 5 pathway model for the mechanism of SHM based on human data.

P.A6.02.15

Whole exome sequencing disclosed heterogeneous gene defects in pediatric SLE

S. Zoghi[1,2,3], *V. Ziaee*[4,5], *E. Salzer*[3,6], *T. Hirschmugl*[3], *R. Jimenez-Heredia*[3], *A. Krolo*[3], *K. Boztug*[3,6,7], *N. Rezaei*[1,2,8];
[1]*Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of,* [2]*Research Center for Immunodeficiencies, Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of,* [3]*Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases, Vienna, Austria,* [4]*Division of Pediatric Rheumatology, Children's Medical Center, Pediatrics Center of Excellence, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of,* [5]*Pediatric Rheumatology Research Group, Rheumatology Research Center, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of,* [6]*St Anna Kinderspital and Children's Cancer Research Institute, Department of Pediatrics, Medical University of Vienna, Vienna, Austria,* [7]*CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria,* [8]*Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Tehran, Iran, Islamic Republic of.*

Systemic Lupus Erythematosus (SLE) is considered as prototypic autoimmune disease and although heavily studied remains enigmatic. SLE is rare in children but if occurs early in life, it is associated with considerably higher morbidity and mortality. In the assumption that in Pediatric SLE (pSLE) a stronger genetic component is expected, we performed exome sequencing in 25 severe, early onset familial pSLE patients. Consanguinity, disease severity and multiple-case families were considered as priority in patient recruitment. Apart from known disease causing complement deficiencies (*C1Q* deficiencies) which have been reported previously, we identified several unexpected disease causing variants to date reported in the context of primary immunodeficiency rather than SLE. While 5 patients showed *C1QA*, *C1QB* and *C1QC* deficiency, mutations in several other genes such as *TREX1*, *PRKCD*, *DNASE1L3* and *ACP5* were identified. In total we could solve 40% of the cases by this approach and out of 25 we had 20% *C1Q* deficiency. Although *C1Q* detection is possible by other methods, sequencing provides a fast and unbiased method to identify disease etiology in SLE patients apart from complement deficiency. Moreover, identification of the molecular diagnosis is crucial for treatment stratification and genetic counselling in these families.

P.A6.02.16

EROS is required for phagocyte NADPH oxidase function in humans and its deficiency causes Chronic Granulomatous Disease.

D. C. Thomas[1], *L. Charbonnier*[2], *A. Schejtman*[3], *H. Aldhekri*[4], *E. Coomber*[5], *E. R. Dufficy*[1], *A. Beenken*[1], *J. Lee*[1], *S. Clare*[5], *A. Speak*[5], *A. Thrasher*[6], *G. Santilli*[6], *H. Almousa*[7], *F. Alkuraya*[8], *T. Chatila*[2], *K. Smith*[1];
[1]*University of Cambridge, Cambridge, United Kingdom,* [2]*Boston Children's Hospital, Harvard University, Boston, United States,* [3]*Institute of Child Health, University College London, London, United Kingdom,* [4]*Department of Paediatrics, KFHSR, Riyadh, Saudi Arabia,* [5]*Wellcome Trust Sanger Institute, Cambridge, United Kingdom,* [6]*University College London, London, United Kingdom,* [7]*Department of Paediatrics, KFSHR, Riyadh, Saudi Arabia,* [8]*Department of Genetics, KFSHRC, Riyadh, Saudi Arabia.*

The phagocyte respiratory burst is mediated by the phagocyte NADPH oxidase, a multi- protein subunit complex that facilitates production of reactive oxygen species and which is essential for host defence. Monogenic deficiency of individual subunits leads to chronic granulomatous disease (CGD), which is characterized by an inability to make reactive oxygen species, leading to severe opportunistic infections and auto-inflammation. However, not all cases of CGD are due to mutations in previously identified subunits. We recently showed that Eros, a novel and highly conserved ER-resident transmembrane protein, is essential for the phagocyte respiratory burst in mice because it is required for expression of gp91phox-p22phox heterodimer, which are the membrane bound components of the phagocyte NADPH oxidase.
We now show that the function of EROS is conserved in human cells. CRISPR-mediated deletion of EROS in human PLB-985 cells abolished expression of gp91phox and p22phox as well as the phagocyte respiratory burst. Both gp91phox-p22phox expression and reactive oxygen species production could be restored to such cells by lentivirally mediated restoration of EROS expression. Further, we describe a case of CGD secondary to a homozygous EROS mutation that abolishes EROS protein expression. This work demonstrates the fundamental importance of EROS in human immunity and describes a novel cause of CGD

P.A6.02.17

Mucosal macrophages express elevated levels of HDAC9 in inflamed and uninflamed mucosa of Crohn's disease, but not ulcerative colitis

M. Ghiboub[1], *J. de Bruyn*[1], *C. Wichers*[2], *T. Radstake*[2], *K. Reedquist*[2], *M. Wildenberg*[1], *J. C. Broen*[2], *D. Geert R*[1], *W. de Jonge*[1];
[1]*Academic Medical Center, Amsterdam, Netherlands,* [2]*Utrecht Univeristy Medical Center, Utrecht, Netherlands.*

Histone-deacetylases (HDACs) are a group of enzymes that control histone/non-histone deacetylation. Certain members of HDAC family control the function of macrophages. We aimed to study the expression of HDACs in mucosal macrophages isolated from inflammatory bowel diseases (IBD) patients.
Macroscopically inflamed and non-inflamed colon resection tissue were collected from 15 Crohn's disease (CD) and 9 ulcerative colitis (UC) patients operated on for therapy refractory disease. Lamina propria was separated from the muscularis externa, and a targeted array for epigenetic enzymes was performed.
From our array, gene expression of HDAC9 in non-inflamed mucosa from CD was elevated compared to non-inflamed mucosa from UC (p=0.005). In addition, in CD, HDAC9 mRNA level was increased in inflamed tissue in comparison to non-inflamed tissue (p=0.046). To assess the relevance of HDAC9 gene expression in terms of protein level, immunofluorescence staining of HDAC9 protein was undertaken in tissue sections from inflamed and non-inflamed mucosa. CD68 was used as a pan-macrophage marker. In conjunction with the expression data, HDAC9 protein was found highly expressed in inflamed tissue. HDAC9 was predominantly localized in the cytoplasmic compartment of macrophages in non-inflamed tissue, whilst HDAC9 localized to the nucleus of macrophages in inflamed tissue. We suggest here, that HDAC9 can serve as an additional marker to distinguish CD from UC in tissue biopsies. Furthermore, we show for the first time that HDAC9 protein is expressed in mucosal macrophages of CD patients, indicating its potential in mediating macrophages function in IBD. Further studies are currently being undertaken to elucidate the role of HDAC9 in CD pathogenesis

P.B1.01 Tumor vaccination principles and Immunotherapy - Part 1

P.B1.01.01

Dichloroacetate and metformin sensitize human tumor cells to the cytotoxic action of NK cells and CTL

J. Marco-Brualla[1], *N. Allende-Vega*[2], *O. Gonzalo*[1], *I. Marzo*[1], *M. Villalba*[2], ***A. Anel***[1];
[1]*University of Zaragoza, Zaragoza, Spain,* [2]*Institute for Regenerative Medicine and Biotherapy (IRMB), Montpellier, France.*

Introduction: Dichloroacetate (DCA), an inhibitor of pyruvate dehydrogenase kinase, forces cells to obtain energy through mitochondrial oxidative phosphorylation and is used in the treatment of lactic acidosis. Metformin is the most common treatment of type II diabetes, and decreases glucose concentration in blood. It inhibits mitochondrial complex I, but has pleiotropic effects, e.g., activation of AMPK.
Materials and Methods: Several leukemic or multiple myeloma cell lines were supplemented for 72h with non toxic concentrations of DCA or of metformin, and then they were used in cytotoxic assays with expanded allogeneic NK cells or cytotoxic T lymphocytes (CTL). Results: DCA sensitizes the human multiple myeloma MM1.S to cytotoxicity exerted by NK cells and by CTL. The combined blocking of NKG2D ligands and LFA-1 abrogates DCA sensitization to CTL without affecting basal cytotoxicity. LFA-1 blocking abrogates NK cell cytotoxicity on MM1.S cells and, in consequence, also DCA sensitization. On the other hand, metformin sensitizes the human B-CLL cell line Mec1 to CTL and especially to NK cells. This sensitization is also observed in Mec1 cells overexpressing the anti-apoptotic proteins Bcl-x_L. LFA-1 blocking, but not NKG2D ligand blocking, abrogates the metformin-induced sensitization to NK cells observed on Mec1 and on Mec1-Bcl-x_L cells. Finally, metformin sensitization to NK cell cytotoxicity on Mec1-Bcl-x_L cells is partially prevented by death receptor blocking. All these data offer a new approach to the improvement of tumor immunotherapy by the combination with metabolic inhibitors.

P.B1.01.02

The influence of tetraspanin CD37 on the expression and distribution of CD20 on lymphoma B cells

A. B. Arp, *C. M. de Winde, M. Bezembinder, E. Jansen, S. J. van Deventer, A. B. van Spriel;
Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboud university medical center, Nijmegen, Netherlands.*

Immune cell function is heavily dependent on the proper localisation of many surface molecules. This localisation is for a large part orchestrated by tetraspanin proteins. This superfamily of 4-transmembrane proteins forms protein clusters to ensure proper distribution of surface receptors needed for immune cell interactions. Tetraspanin CD37 is exclusively expressed by immune cells, with its highest expression on B cells. Recent findings of our group show a critical role for CD37 expression in the clinical outcome of patients suffering from aggressive B cell lymphoma.
The standard treatment for B cell lymphoma is a combination therapy using chemotherapy (CHOP) and the anti-CD20 antibody Rituximab. Patients with CD37-negative lymphoma respond less to this combination therapy, indicating that CD37 controls expression and/or distribution of CD20 on the cell surface. This is supported by preliminary data showing that lymphoma cell lines lacking CD37 often also lack CD20 expression. Importantly, these B cell lines are less responsive to Rituximab treatment when compared to CD37-positive lymphoma cell lines.
We aim to elucidate the role of CD37 in the expression, distribution and localisation of CD20 on healthy B cells, lymphoma cell lines and patient lymphoma cells. We established an extensive array of aggressive B cell lymphoma models in which CD37 expression is manipulated by either overexpression or complete knock-out using the CRISPR/Cas9 technique. This study not only provides fundamental insights into how CD37 organises the B cell membrane, but also contributes to the development of new immunotherapies for patients with aggressive B cell lymphoma.

P.B1.01.03

Melanin is superior to other adjuvants to trigger CD8 T cells in subunit vaccines

*C. Banissi[1], F. Sejalon[1], T. Tran[2], E. Tartour[2], **A. F. Carpentier**[1];*
[1]Hopital Saint Louis, Paris, France, [2]INSERM U970, Paris, France.

Introduction: Synthetic melanin bound to subunit vaccine antigens enhances CD8+ T-cell responses in mice, when combined with a TLR9 agonist. We here compared the efficacy of various vaccine adjuvants, alone or in combination with melanin, to trigger CD8 T-cell responses. Material and Methods: Two peptides containing a gp100 or an ovalbumine epitope were mixed with L-Dopa and further oxidized to generate melanin-bound peptides. Different TLR9 agonists (CpG-28, 1826, ISS), polyinosinic-polycytidylic acid (Poly-IC), Freund adjuvant (v/v), or aluminium hydroxide (Alum, v/v) were used as adjuvants in vaccine formulations. C57BL/6 mice were immunized subcutaneously (n=8/group), and the CD8 immune response was assessed with epitope-specific IFNγ production by splenocytes. Results: When Alum was used as an adjuvant, no significant CD8 response was seen, even with melanin-bound peptides. With Freund adjuvant, combined or not with a TLR9 agonist, a mild CD8 response was seen using either free or melanin-bound peptides. On the contrary, both TLR9 agonists and poly-IC elicited a CD8 T-cell response with free peptides, and this response was several fold enhanced when melanin-bound peptides were used, making this combination an order of magnitude greater than any others. Analysis of dextramer$^+$ CD8$^+$ cells showed an effector memory CD8$^+$ T-cells phenotype. The minimal dose of peptides required to trigger immunity was 0.5 µg. Immunization against the ovalbumin epitope inhibited the growth of ovalbumin-positive tumors. Conclusion: The conjugation of synthetic melanin to peptides represents a very simple means of triggering CD8 T-cell response, which should be particularly useful in cancer immunotherapy against neo-epitopes.

P.B1.01.04

Immune response induced in mice by codelivery of STING recombinant and HPV DNA vaccine

*W. Zhang[1], D. Chen[2], Z. Wang[2], **J. Chen**[2];*
[1]Clinical Laboratory,Linan People's Hospital, Hangzhou, China, [2]Institute of Immunology,School of Medicine,Zhejiang University, Hangzhou, China.

Cervical cancer is the second most common cancer among women worldwide and remains a clinical problem despite improvements in early detection and therapy. HPV DNA vaccines have become an attractive approach to treat HPV-related cancer. To investigate the effect of STING in immune response induced by plasmid encoding HPV E6E7 protein and to explore new strategies for prophylactic and therapeutic HPV DNA vaccines, C57BL/6 mice and TC-1 tumor cell bearing mice were immunized with pVAX-E6E7 alone or co-immunized with pVAX-STING. The specific CTL response and Th1 cell response were also assayed. The co-immunization of pVAX-STING and pVax-E6E7 significantly inhibited the growth of TC-1 after immunization and prevent the TC-1 tumor cell bearing mice from developing into tumor compared with the mice immunized with pVax-E6E7 alone. Furthermore, the immunological mechanism behind immune enhancing effect of STING can be attributed to increased cytotoxic T lymphocyte, accompanied by the up-regulation of Th1-cytokine IFN-γ. The result showed that co-administration of STING could elicit stronger immune response induced by HPV DNA vaccines and It provided scientific basis for the further use of STING as a HPV DNA vaccine adjuvant. This work was supported by grants from Zhejiang Provincial Natural Science Foundation of China (No. LY14H100003), Science and Technology Department of Zhejiang Province(No.2016C37121) and Health and Family Planning Commission of Zhejiang province (No.2016KYB032)

P.B1.01.05

Comparing the efficiency of two clinical grade stimuli on BDCA3 mDCs by using transcriptomics

*T. S. Mathan[1], **G. Flórez-Grau**[1], T. van Oorschot[1], S. I. Buschow[2], G. Schreibelt[1], I. Reinieren-Beeren[1], D. Sancho[3], C. Alfaro[4,5,6], C. G. Figdor[1], I. J. M. de Vries[1,7], J. Textor[1];*
[1]Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboudumc, Nijmegen, Netherlands, [2]Department of Gastroenterology and Hepatology, Erasmus MC-University Medical Center, Rotterdam, Netherlands, [3]entro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain, [4]Division of Gene Therapy and Hepatology, Centre for Applied Medical Research (CIMA, Pamplona,, Spain, [5]Department of Oncology, University Clinic of Navarra,, Pamplona, Spain, [6]Department of Immunology, University Clinic of Navarra, Pamplona, Spain, [7]Department of Medical Oncology, Radboud Institute for Molecular Life Sciences, Radboudumc, Nijmegen, Netherlands.

Maturation of dendritic cells (DC) is considered critical in cancer immunotherapy. Among different human subsets, BDCA-3 (CD141high-Clec9A$^+$), promote CD8 T-cell cross-priming against tumor antigens. Here, we evaluate two clinical grade stimuli for peripheral blood BDCA3 myeloid dendritic cells (mDCs), a rare DC subset that is currently being explored for use in immunotherapy. We applied an unbiased transcriptome-based method using both RNA-sequencing (RNA-seq) and microarraysIn particular, we analyzed the mRNA of human BDCA3$^+$ mDCs upon activation with two clinical-grade adjuvants, Hiltonol (poly ICLC, a TLR3 ligand) and protamine RNA (pRNA, aTLR7/8 ligand), and compared the data to unstimulated counterparts. Our results, based on both RNA-seq and microarray data, showed that Hiltonol and pRNA lead to almost identical changes in BDCA3 mDCs, both at the transcriptome and at protein levels. The gene ontology (GO) term analysis suggests that these changes were mainly related to activation and maturation pathways, including induction of type I IFN and IL-12 transcription, while pathways related to adverse effects or cell damage were not significantly affected. The combination of both stimuli in the DC cultures gave a very similar result as compared to either stimulus alone, suggesting no synergistic effect. To sum up, our results indicate that both Hiltonol as well as protamine mRNA are equally potent clinical grade adjuvants with comparable effects on BDCA3 mDCs after short-term culture. This paves the way for introducing BDCA3 mDCs into a clinical setting.

P.B1.01.06

Anti-tumoral potential of granulysin-containing protein products directed against the Tn antigen*

***P. Guerrero**[1], R. Ibáñez[1], F. Corzana[2], R. Hurtado-Guerrero[3], A. Anel[1];*
[1]University of Zaragoza, Zaragoza, Spain, [2]La Rioja University, Logroño, Spain, [3]Institute for Biocomputation and Physics of Complex Systems (BIFI), Zaragoza, Spain.

Introduction: Granulysin is a protein present in the granules of human CTLs and NK cells, with cytolytic activity against microbes and tumors. Previous work from our group demonstrated the *in vivo* antitumoral activity of intratumoral injection of recombinant granulysin in two animal models of tumor development, breast adenocarcinoma and multiple myeloma. In the present work we have developed two granulysin-containing protein products directed against the MUC1 Tn antigen, expressed in several types of solid tumors. Material and Methods: 1) Recombinant granulysin or the protein products were tested for binding to cells negative for the MUC1 Tn antigen expression (MDA-MB-231), or to Tn$^+$ cells (Jurkat and MCF7) by flow cytometry and fluorescence microscopy. 2) Recombinant granulysin or the protein products were tested against cells negative for Tn expression (MDA-MB-231) or on Tn$^+$ cells (Jurkat and MCF7) and cell death was analyzed by annexin-V staining and 7-AAD incorporation. Results: We demonstrated that the protein products recognized the Tn antigen in an specific fashion on Tn-positive tumor cells. In addition, the bioactivity of the protein products against tumor cell lines expressing Tn is higher than that of granulysin alone. *The "protein products" are under patent tramitation

P.B1.01.07

A novel marker for M2-polarization: Migration Stimulating Factor promotes monocyte chemotaxis

*T. Gulic[1,2], I. Laface[1], A. Inforzato[1,3], M. Oliveira[4], F. Petroni[1], S. Valentino[1], S. Davudian[1], P. Allavena[1], **B. Bottazzi**[1], A. Mantovani[1,3,5];*
[1]Humanitas Research Hospital, Milan, Italy, [2]Medical Faculty, Rijeka, Croatia, [3]Humanitas University, Milano, Italy, [4]4i3S- Institute of Inovation and Research, University of Porto, Porto, Portugal, [5]The William Harvey Research Institute, Queen Mary University of London, London, United Kingdom.

Chronic inflammation is a well-known key component in preparing tumour microenvironment to acquire all the hallmark capabilities for tumour growth.Besides neoplastic cells, the tumor-associated macrophages (TAMs) are the principal leukocyte subset in tumour microenviroment directly affecting different biological activities, including neoplastic cell growth, neoangiogenesis, and extracellular matrix remodelling. Increasing evidences correlated the levels of tumor-associated macrophages (TAMs) with bad prognosis. We recently identified in M2 polarized macrophages up-regulation of a set of genes among which is Migration Stimulating Factor (MSF),a truncated isoform of fibronectin 1 initially described in fetal fibroblasts and tumor cells. Here we report that MSF was induced mostly by M-CSF, IL-4 and IL-10 but not by proinflammatory stimuli. RNA analysis clearly demonstrated that it is selectively associated with the M2 polarization of macrophages. We developed original reagents (recombinant protein, monoclonal antibodies) for MSF study. MSF production was confirmed by immunohistochemistry and immunofluorescence in human tumour cells and TAMs. Double immunostaining indicated that most of the CD68, CD206 and CD163 positive cells expressed MSF. rhMSF strongly stimulated tumor cells, monocytes and possible neutrophils migration, while it did not affect motility of NK, CD4$^+$and CD8$^+$cells.Checkerboard analysis revealed MSF as a gradient-dependent chemotactic molecule for human monocytes.These results suggest that MSF may facilitate monocyte extravasation and migration, a crucial event into inflame tissue. Modulating monocyte migration, MSF might promote and/or sustain a permissive microenvironment for cancer growth.

P.B1.01.08

Intra-tumoral production of IL18, but not IL12, by TCR-engineered T cells is non-toxic and counteracts immune evasion of solid tumors

***A. Kunert**[1], M. Chmielewski[2], R. Wijers[1], C. Berrevoets[1], H. Abken[2], R. Debets[1];*
[1]Erasmus MC Cancer Institute, Rotterdam, Netherlands, [2]University Hospital Cologne and Center for Molecular Medicine Cologne, Cologne, Germany.

Adoptive therapy with engineered T cells shows promising results in treating patients with malignant disease, but is challenged by incomplete responses and tumor recurrences. Here, we aimed to direct the tumor microenvironment in favor of a successful immune response by local secretion of interleukin (IL-) 12 and IL-18 by administered T cells. We engineered T cells with a melanoma-specific T cell receptor (TCR) and murine IL-12 and/or IL-18 under the control of a nuclear-factor of activated T-cell (NFAT)-sensitive promoter. These T cells produced IL-12 or IL-18, consequently enhancing levels of IFNγ, following exposure to antigen-positive but not negative tumor cells. Adoptive transfer of T cells with a TCR and inducible (i)IL-12 to melanoma-bearing mice resulted in severe, edema-like toxicity accompanied by enhanced levels of IFNγ and TNFα in blood, and reduced numbers of peripheral TCR transgene-positive T cells. In contrast, transfer of T cells expressing a TCR and iIL-18 was without side effects, enhanced the presence of therapeutic CD8+ T cells within tumors, reduced tumor burden and prolonged survival.

Treatment with TCR+iIL-12 but not iIL-18 T cells resulted in enhanced intra-tumoral accumulation of macrophages, accompanied by a decreased frequency of therapeutic CD8+ T cells. In addition, when administered to mice, iIL-18 but not iIL-12 demonstrated a favorable profile of T cell co-stimulatory and inhibitory receptors. In conclusion, we observed that treatment with T cells engineered with a TCR and iIL18 T cells is safe and able to skew the tumor microenvironment in favor of an improved anti-tumor T cell response.

P.B1.01.10

IL-12-dependent Th1 priming by a DC vaccine in vivo requires cooperation of dendritic cells subsets

*D. Ashour, **M. B. Lutz**;*
University of Würzburg, Würzburg, Germany.

The production of heterodimeric IL-12p70 by injected vaccine dendritic cell (DCs) has been classically described as a key factor required for generating polarized T helper type-1 cell response (Th1). However, cocktail-matured DCs do not secrete IL-12 but readily induce Th1 responses when injected into mice and humans. Here, we tested for DC-DC cooperation enabling bystander IL-12 production for Th1 polarization in a DC vaccination model. Subcutaneously injected LPS-matured and OVA peptide loaded bone marrow-derived DCs (BM-DCs) migrated to the draining lymph node in the recipient mice and induced responder CD4+ OT-II T cells to polarize into Th1 cells. Injected CCR7-/- DCs, which lack migratory capacity, failed to induce OT-II cell priming and Th1 induction, indicating that the injected DCs provide the peptide presentation and costimulation. DC vaccination increased also the migration of all endogenous skin-resident DC subsets to the draining lymph nodes. However, only the $CD103^+$ dermal DCs showed increased IL-12p40-YFP production culminating at 72h after BM-DC injection. Surprisingly, injections of BM-DCs derived from p35-/- mice did not affect Th1 priming, while deficiency of p35 in the recipient mice abrogated the IL-12-dependent Th1 priming, which indicates that IL-12 production by endogenous DCs and not the injected BM-DCs is required for Th1 priming in this DC vaccine model in the draining lymph node. Further studies will show the requirement of IL-12 from $CD103^+$ dDCs for Th1 priming. Together, our data indicate that DC vaccines require a cooperation with endogenous DCs for optimal Th1 polarization of CD4+ T cells.

P.B1.01.11

Gene-edited chimeric antigen receptor (CAR) T cells: Tuning up for the next generation cancer immunotherapy

***H. R. Mirzaei**[1], F. Raghami[2], H. Mirzaei[2];*
[1]Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of, [2]Isfahan University of Medical Sciences, Isfahan, Iran, Islamic Republic of.

Recently clinical trials utilizing genetically engineered T cells expressing a chimeric antigen receptor (CAR) that is half monoclonal antibody and half T-cell receptor have demonstrated remarkable response in patients with advanced cancers like relapsed or refractory acute lymphoblastic leukemia (ALL) and lymphoma. Moreover, emerging chimeric genome editing tools such as zinc-finger nucleases (ZNFs), transcription activator-like effector nucleases (TALENs) and clustered regulatory interspaced short palindromic repeat (CRISPR)/Cas composed of sequence-specific DNA binding module(s) linked to a non-specific DNA cleavage domain have made possible to dramatically expand the ability to manipulate cells aim to treat and/or study a wide range of diseases including cancer. Here, we will discuss how joint application of these two chimeras will help us to manipulate CAR T cells aiming to enhance the efficacy of CAR T cell therapy in preclinical and clinical settings.

P.B1.01.12

Effects of ionizing radiations on T and B lymphocytes: a comparison between photons and protons

*F. Novelli[1], M. Vadrucci[2], M. M. Rosado[3], L. Picardi[2], C. Ronsivalle[2], E. Benvenuto[4], C. Marino[1], **C. Pioli**[1];*
[1]ENEA, Division of Health Protection Technologies, Rome, Italy, [2]ENEA, Laboratory of Development of Particle Accelerators and Medical Applications, Rome, Italy, [3]Freelance Research Consultant in Immunology, Rome, Italy, [4]ENEA, Laboratory of Biotechnology, Rome, Italy.

Photon radiotherapy (γ/X-rays) is widely used to treat a large variety of cancers, while particle-based radiotherapy (protons or carbon ions) represents a developing and valuable option, especially for cancers requiring more focused treatments. Indeed, proton beams display low entrance dose, uniform high dose on targeted tumor (spread-out-Bragg peak) and near zero dose beyond it, thus preserving not targeted tissues. Recent studies indicated that photon and particle radiations produce different biological effects, with relevant outcomes once combined as radio/immune-therapies.
We compared the effects of local *in vivo* exposure (2 Gy) to medium energy proton and X-ray beams on mouse lymphoid spleen cells. Proton irradiation was carried out with the 27 MeV beam produced by the TOP-IMPLART pulsed linear accelerator. For X-rays, a CHF320G generator (250 kV, 15 mA) was used. During the exposure, mice (C57Bl/6) were anesthetized; not targeted areas were protected by shields. At different time points after irradiation (1-28 days), mice were analyzed for number and phenotype of different T (CD4/CD8/Foxp3) and B (IgM/IgD) cell subsets, B and T cell proliferation, cytokine production (IL-2, IFNγ, IL-4, IL-17, IL-6, TNF, IL-10), and antibody production (IgM, IgG). Altogether, the results showed that local exposure to X-rays or protons induced changes in many of the analyzed parameters, with different effects and recovery time depending on the type of radiation used.
This research was partially supported by the BIOxTREME (Italian Space Agency) and the TOP-IMPLART (Regione Lazio, Italy) projects.

P.B1.01.13

Activation of murine splenocytes against tumor cells by sensitization with babassu mesocarp

***L. P. Pontes**, J. G. Junior, E. A. Moraes, M. C. Pinto, G. C. Costa, R. N. Guerra, F. R. Nascimento, A. S. Santos;*
Federal University of Maranhão, São Luis, Brazil.

Introduction: *Attallea speciosa* Mart. (babassu) fruit contains a mesocarp that is rich in carbohydrates with immunomodulatory effects. The induction of the tolerogenic response is a tumor escape mechanism, and immunomodulator adjuvants have been studied to reestablish host immunogenicity. This study evaluates the adjuvant potential of babassu mesocarp carbohydrates in a tumor model. Materials and Methods: The polysaccharide obtained from aqueous babassu extract (20 mg/ml) was used tosensitizationthe animals inoculated or not with the tumor to obtain splenocytefor the phenotypic characterization and lymphoproliferation assay. Results:The babassu mesocarp extraction (BME) yield was 75.54%, and the total sugar concentration was 29.79 mg/ml-1 containing monosaccharides, reducing sugars, polysaccharides and 0.506 mg/ml-1 total protein. Chromatography analysis identified glucose, sucrose and fructose. Sensitization increased the spleen weight in the tumor group compared with the control, and a comparatively lower frequency of T helper and higher frequency of B-lymphocytes was also observed. The tumor+BME group had more cytotoxic T lymphocytes compared with the control. After co-culture with cancer cells, the tumor splenocytes showed lower proliferation, lower frequency of T helper cells and higher concentrations of interleukin (IL)-2, IL-6 and IL-10. However, the tumor+BME splenocytes presented results similar to the control, suggesting a reduction in the regulatory response of the tumor group. Conclusions:These results demonstrated that BME sensitization with cancer cells modulated an immune response in Balb/c animals, indicating an immunogenic effect. Key words: Arecaceae, *Attallea speciosa* Mart., adjuvant, carbohydrate, antitumor.

P.B1.01.14

HPMA copolymer-bound doxorubicin as an endogenous vaccine substantially increases the therapeutic effects of check-point blockade monoclonal antibodies

***B. Rihova**[1], T. Etrych[2], R. Stepankova[3], V. Subr[2], K. Ulbrich[2], M. Kovar[3], M. Sirova[3];*
[1]Institute of Microbiology AS CR, Prague, Czech Republic, [2]Inst. Macromolecular Chemistry AS CR, Prague 6, Czech Republic, [3]Institute of Microbiology AS CR, Prague 4, Czech Republic.

DOX^{HYD}-pHPMA is doxorubicin bound to a synthetic polymeric carrier based on *N*-(2-hydroxypropyl)methacrylamide via a pH-sensitive hydrazone bond. It is effective anticancer polymeric prodrug with decreased side-toxicity and the ability to induce immunogenic cancer cell death releasing tumor antigens and thus acting as endogenous vaccine. We investigated a novel combination strategy using low dose of DOX^{HYD}-pHPMA and immune checkpoint blocking anti-CTLA-4 and anti-PD-1 mAbs to treat EL4 T cell lymphoma and 4T1 breast carcinoma. Acute model of disease when mice are transplanted once with a lethal dose of tumor cells was compared with chronic model where mice are injected six times every other day with a low number of tumor cells. Significant toxicity of anti-CTLA-4 and anti-PD-1 mAbs was seen in mice suffering from acute disease. Mice with chronic cancer respond to treatment much better than those with acute disease. Therapy with anti-CTLA-4 and/or with anti-PD-1 only led to twenty-five percent survival of the treated mice, which were considered long-term-survivors (LTS). On the other hand more than 60% of mice injected also with therapeutically suboptimal dose of DOX^{HYD}-pHPMA survived disease-free for more than 100 days. In germ-free mice, tumor grow was more aggressive, and the survival time was shorter. Treatment with DOX^{HYD}-pHPMA showed only limited anti-cancer effects while the combination with the anti-CTLA-4 monoclonal antibody significantly improved the therapeutic outcome. The best results were seen in germ-free mice monocolonized with *B. thetaiotaomicron.* This work was supported by the Ministry of Health CR (grant number 16-28600A) and the CSF (grant number 17-08084S).

P.B1.01.15

PD-L1 expression according to five monoclonal antibodies in urothelial cell cancer: concordance and clinical implications

M. Rijnders[1], *A. van der Veldt*[1], *T. Zuiverloon*[1], *J. Boormans*[1], *E. Zwarthoff*[1], *K. Grünberg*[2], *E. Thunnissen*[3], *M. Lolkema*[1], *R. Debets*[1], *R. de Wit*[1], *A. van Leenders*[1];
[1]*Erasmus MC, Rotterdam, Netherlands,* [2]*Radboudumc, Nijmegen, Netherlands,* [3]*VUmc, Amsterdam, Netherlands.*

Introduction High PD-L1 expression is frequently applied as an inclusion criterion or stratification factor in clinical trials on immune checkpoint inhibitors (ICIs). However, the predictive value of PD-L1 in urothelial cell cancer (UCC) shows conflicting results, which may be confounded by the use of different PD-L1 companion diagnostics. The objective of this study was to accurately compare PD-L1 expression of five commercially available PD-L1 antibodies in UCC patients. **Methods** Tissue Microarrays (TMA) containing samples of 141 muscle-invasive UCC patients (≥pT2) were stained with the anti-PD-L1 antibodies 22C3, 28-8, SP142, SP263 and E1L3N on the Ventana Benchmark (SP142, SP263) and DAKO platforms (22C3, 28-8, E1L3N). PD-L1 expression was manually scored on tumor cells and infiltrating immune cells according to corresponding assay specifications used in clinical trials. **Results** PD-L1 expression was found to be positive in 20% (SP263), 21% (SP142), 23% (28-8), 27% (22C3), and 27% (E1L3N) of cases. No relations between clinicopathologic parameters and PD-L1 expression were observed. Concordance in treatment-determining score varied from 72% to 90% and was lowest for E1L3N (mean 75%). Considering only companion diagnostic tests 22C3, 28-8, SP142 and SP263, PD-L1 status was concordant in 78% of patients. When one test result was discordant (n=15; 11%), SP142 (n=7) and 28-8 (n=5) were most likely different. **Conclusion** Agreement of PD-L1 assessment is good with similar PD-L1 status by four antibodies used in companion diagnostic tests. Therefore, application of different companion PD-L1 antibodies and platforms may have limited effects on therapeutic decision making in ICI treatment.

P.B1.01.16

Oncolytic adenovirus coding for TNFa and IL-2 removes the need for lymphodepleting preconditioning in adoptive T-cell therapy

J. Santos[1,2], *V. Cervera-Carrascon*[1,2], *R. Havunen*[1,2], *S. Zafar*[2], *M. Siurala*[1], *S. Sorsa*[1], *M. Anttila*[3], *A. Hemminki*[1,2,4];
[1]*TILT Biotherapeutics Ltd, Helsinki, Finland,* [2]*Cancer Gene Therapy Group, Helsinki, Finland,* [3]*Finnish Food Safety Authority (EVIRA), Helsinki, Finland,* [4]*Helsinki University Hospital Comprehensive Cancer Center, Helsinki, Finland.*

Introduction: Lymphodepleting preconditioning with high-dose chemotherapy remains a critical component for the clinical effectiveness of several adoptive T-cell therapy (ACT) strategies. This preconditioning step boosts the antitumor efficacy of transferred T cells through the decrease of tumor immunosuppression, however, with severe toxicity for patients. In contrast, oncolytic adenoviruses are safe and when engineered to express interleukin-2 (IL-2) and tumor necrosis factor alpha (TNFa), they can achieve immunomodulatory effects similar to lymphodepleting preconditioning.
Materials and Methods: Here, we compare the safety and efficacy of such adenovirus with a cyclophosphamide and fludarabine lymphodepleting regimen in the context of ACT. Since Syrian hamsters allow replication of a human adenovirus (Ad5/3-E2F-D24-hTNFa-IRES-hIL2; TILT-123), we used a pancreatic tumor model (HapT1) in syngeneic hamsters infused with tumor infiltrating lymphocytes (TIL). To study immune cells responsive to TNFa and IL-2, we used an immunocompetent mouse melanoma model (B16.OVA) infused with ovalbumin-specific T cells.
Results: Animals receiving oncolytic adenovirus therapy demonstrated better tumor growth control and survival compared with those receiving lymphodepleting preconditioning. Moreover, the adenovirus approach increased the levels of Th1-type cytokines and infiltration CD3+ T cells and CD11c+CD86+ dendritic cells. While lymphodepleting preconditioning resulted in severe toxicities in the heart and lungs, adenovirus therapy caused minimal changes in treated animals.
Conclusion: Overall, this data shows that ACT protocols using oncolytic adenovirus expressing IL-2 and TNFa do not require high-dose preconditioning chemotherapy. Clinical translation is ongoing in a Phase I clinical trial where melanoma patients administered with TIL therapy receive TILT-123 instead of lymphodepleting chemotherapy.

P.B1.01.17

Tumor-type-specific spatio-temporal shifts of lymphoid and myeloid populations during tumor growth and checkpoint blockade

S. T. T. Schetters*, L. J. Kruijssen, M. H. Crommentuijn, Y. Van Kooyk;*
VU University Medical Center, Amsterdam, Netherlands.

Suppression of the immune system by solid malignancies has proven to be a driving force of tumor development and an effective target for therapeutic intervention. Especially the suppression of cytolytic T cells through inhibitory receptors, like PD-1 and CTLA-4, can be blocked by antagonistic antibodies, reinvigorating existing anti-tumor responses. However, it unclear whether immune checkpoint interactions are heterogeneous within the tumor, how these interactions develop during tumor growth and which cell types interact in the tumor microenvironment. By using high dimensional flow cytometry and unsupervised clustering analyses based on immune checkpoints, we show heterogeneity of tumor-infiltrating $CD8^+$ and $CD4^+$ T cells in murine B16 melanoma and MC38 colorectal carcinoma. Also, we show that the myeloid- and tumor cell compartments provide the ligands for immune checkpoint suppression. Next, we show that therapeutic intervention using anti-PD1 treatment, changes lymphoid and myeloid populations and existing immune checkpoint interactions within the tumor. Finally, we reconstruct the tumor microenvironment using 8-color confocal microscopy and histocytometry analysis to spatially reconstruct the changing immune compartments of the tumor microenvironment.

P.B1.01.18

Human Monoclonal IgGs derived from patients with Multiple Myeloma are able to penetrate living neoplastic cells and induce apoptosis

T. Stivarou[1], *I. Sarrigeorgiou*[1], *P. Chalas*[1], *A. Tsirogianni*[2], *P. Lymberi*[1], ***C. Tsigalou***[2];
[1]*Immunology Laboratory, Immunology Dept. Hellenic Pasteur Institute, Athens, Greece,* [2]*Immunology-Histocompatibility Dept. "Evaggelismos" General Hospital, Athens, Greece.*

Introduction:Antibodies able to penetrate living cells (CPAbs) have been well characterized in patients with SLE and in mouse models. Our lab has described the existence of CPAbs in health, derived either from unimmunized BALB/c mice or from intravenous immunoglobulin (IVIg).The development of human monoclonal IgG-CPAbs is of major importance in drug delivery and in cancer immunotherapy. The aim of the present study was to identify such mAbs among serum monoclonal immunoglobulins (M-IgG) from patients with Multiple Myeloma (MM), and further study their intracellular biological functions in neoplastic cells.
Methods:71 sera from IgG-MM patients were studied by in-house ELISAs against 7 self & non-self antigens.We purified 6 IgG, 5 polyreactive & 1 non-polyreactive by protein-G affinity chromatography and tested them in optimum conditions for ability to: 1) penetrate FcγR+ (Raji & MDA-MB-231) and FcγR- (NIH-3T3 & HeLa) cells by immunofluorescence,2) induce apoptosis on these cells by flow cytometry, and 3) hydrolyze plasmid &genomic DNA.
Results:The 5 purified M-IgGs tested were able to penetrate all cells, either FcγR+ or FcγR-, at 37oC in a dose- and time-dependent mode of entry, while 3/5 also penetrated cells at 4oC (energy-independent entry). All M-IgGs were polyreactive, accumulated in the cytoplasm, induced apoptosis especially in MDA-MB-231 cells, and hydrolyzed plasmid DNA isoforms(1/5 also hydrolyzed genomic DNA).
Conclusion:The IgG-MM sera represent an excellent source of human mIgGs exhibiting cell-penetrating ability and intracellular functionality, and can be exploited as a potential therapeutic tool, used either per se, or as carriers for intracellular drug delivery, or even both.

P.B1.01.19

Highly specific targeting of human acute myeloid leukaemia (AML) cells using functionalised gold nanoparticles

I. M. Yasinska[1], *B. F. Gibbs*[1], *R. Hussain*[2], *G. Siligardi*[2], *E. Fasler-Kan*[3], *L. Calzolai*[4], *V. V. Sumbayev*[5];
[1]*University of Kent, Chatham Maritime, United Kingdom,* [2]*Diamond Light Source, Didcot, United Kingdom,* [3]*University Hospital Bern, Inselspital, Bern, Switzerland,* [4]*European Commission Joint Research Centre, Ispra, Italy,* [5]*University of Kent and Greenwich, Chatham Maritime, United Kingdom.*

Highly specific targeting of human malignant cells with the purpose of recognition and delivery of specific drugs into them is a very promising but not well developed complex of diagnostic and therapeutic strategies. It is a major focus of current molecular cancer research, Immunology and Nanomedicine. In this study we demonstrated for the first time a new approach for highly specific targeting of human acute myeloid leukaemia (AML) cells by functionalised gold nanoparticles carrying single-chain antibody against the immune receptor Tim-3 and rapamycin. Tim-3 is highly expressed in human AML cells. It is one of the key components of Tim-3/galectin-9 secretory pathway which is crucial for survival of malignant cells since it determines their ability to escape host immune surveillance. Thus, Tim-3 can be used as a target for specific recognition of AML cells. Rapamycin inhibits activity of mammalian target of rapamycin (mTOR), a master regulator of translational pathways in AML cells. Inhibiting the mTOR leads to a rapid killing of AML cells. Using these nanoconjugates we managed to successfully deliver rapamycin into the AML cells reaching attenuation of the mTOR activity. Concentration of rapamycin required to reach such an effect is at least 50 times lower compared to the one of free rapamycin required to achieve similar effect. We therefore concluded that our technology is of potential use for highly specific targeting of AML cells for the purpose of diagnosis and possibly therapy. The nanoconjugates can be used to specifically identify malignant blood cells thus allowing rapid AML diagnosis.

P.B1.01.20

Exosome mediated protective cancer vaccine: in vivo performance

M. Yildirim[1], *I. Evcili*[1], *N. Bozbeyoglu*[1], *G. G. Kaya*[1], *G. Aykut*[1], *G. Gucluler*[2], *A. Kamacıoglu*[1], *G. Alıskan*[3], *I. Gursel*[1];
[1]*Bilkent University-Department of Moleculer Biology and Genetics, Ankara, Turkey,* [2]*2Immunology and Allergy Unit, Department of Medicine Solna, Karolinska Institute, and Karolinska University Hospital, Stockholm, Sweden,* [3]*3Depertmant of Biological Sciences, Middle East Technical University, Ankara, Turkey.*

Exosomes are naturally occurring nanosized vesicles that have attracted considerable attention as drug delivery vehicles. Recently, nanoparticles were explored extensively for drug delivery applications aiming to obtain better therapeutic read-outs due to their low toxicity and biocompatibility. Herein, we describe a simple method to externally load magnetic iron oxide nanoparticles (FeO-NPs) along with TLR ligands within exosomes. The internalization and immunostimulatory activities of unloaded or TLR3, TLR9 ligand and FeO-NP loaded exosomes were analyzed either on RAW264.7 murine macrophage-like cell line or on splenocytes at various time and dose intervals. Furthermore, therapeutic efficacy of exosomes loaded with FeO-NPs and TLR ligands (i.e. Exo(pI:C+CpG+FeO))were tested on hu-HUH7-bearing athymic mice. Our data showed that FeO-NPs loaded exosome (Exo(FeO)) uptake by RAW264.7 and splenocytes enhanced up to 10-fold and 3-fold, respectively compared to unloaded exosomes. Moreover, co-encapsulation of CpG with FeO-NPs (Exo(CpG+FeO)) within exosomes significantly magnified CpGODN internalization by RAW 264.7 macrophages compared to FeO-NP devoid CpG ODN loaded exosomes (Exo(CpG)). As expected, Exo(CpG+FeO) treated splenocytes secreted higher amounts of IL-12 compared to Exo(CpG). Lastly, mice were xenotransplanted with HUH7 cells and palpable tumor formation was formed. Tumor-bearing animals that were treated with Exo(pI:C+CpG+FeO)(3x injections @3day intervals) significantly regressed tumor development compared to free mixture treated group. When exosomal therapeutic vaccine was administered there were >70% tumor mass reduction. In conclusion, this study implicated that loading FeO-NPs and TLR ligands within exosomes offers an effective theranostic approach for developing targeted exosomes that could boost their therapeutic impact.

P.B1.01.21

Implantable, pre-activated microconed-Si scaffold vaccines for cancer therapy

I. Zerva[1], *C. Lanara*[2], *E. Stratakis*[2], *I. Athanassakis*[1];
[1]*Department of Biology, University of Crete, Heraklion, Greece,* [2]*FORTH, Heraklion, Greece.*

Therapeutic vaccines are an active immunotherapy of cancer selection aimed at the patient's therapy using immune system of the patient. Over the years, the lack of effective active immunotherapies for cancer have led to the development of many new strategies. One of the major problems is the failure of development of immune responses against tumor antigens since these are usually recognized as antigens themselves. It has been shown that new tumor-specific antigens is an approximate time-consuming, costly and of limited effectiveness. Previous studies have shown that implantable microstructured 3-dimensional scaffolds can support the adhesion of macrophages and after implantation in vivo causes the necessary inflammatory reaction in the body accompanied by secretion of specific antibody, and development T- and B-cell memory. The proposed investigation is the use of the technology of silicon Scaffold in development of an immune response against cancer cells of the host. The study concludes the experimental breast cancer model in Balb/c mice. After reaching the carcinogenic 4T1 cells, the animals implanted with a pre-activated silica scaffolding to which the antigen is the cell histocompatible 4T1 cell extract. This method allows the natural selection of immunogenic epitopes for the development of specific cellular and humoral response against the tumor. The purpose of the research is to regulate the tolerance balance/host immunity and the development of individualized specific response against the tumor. The application of such technology to humans will be of great importance opening novel areas of research and treatment.

P.B1.02 Tumor vaccination principles and Immunotherapy - Part 2

P.B1.02.01

Cessation of thymic activity impairs pTreg differentiation and enhances spontaneous and therapeutic tumor immune-surveillance

J. Almeida-Santos*, M. Bergman, I. Cabral, I. Caramalho, J. Demengeot;*
Instituto Gulbenkian de Ciência, Oeiras, Portugal.

It is well established that depletion or inhibition of regulatory T cells (Treg) in mice and humans, favors immune rejection of solid tumors. While it has been proposed that both thymic- and peripherally-derived Treg (tTreg and pTreg, respectively) can infiltrate the tumor environment, their relative contribution to tumor progression is still unclear. As recent thymic emigrants (RTE) have been shown to be the preferential precursors of pTreg in specific assays, we hypothesize that ongoing thymic activities play a role in tumor immune tolerance.
To test this hypothesis, we assessed whether: i) the higher capacity of RTE to convert into pTreg is also true in a tumor context; ii) newly converted pTreg are required for tumor progression; and iii) thymectomy improves tumor immuno-therapies.
By performing adoptive transfer experiments into lymphopenic hosts, and testing several tumor models, we evidence that immature CD4 cells remain the preferential precursors of pTreg in a tumor context. Using genetically (DEREG) and surgically (Thymectomy) engineered mice to control Treg and RTE numbers in the periphery, we demonstrate that while depletion of Treg limits tumor growth, prevention of pTreg generation through elimination of RTE amplifies this effect. Moreover, we show that limiting thymic activities through thymectomy enhances the efficacy of anti-CTLA-4 immunotherapy.
In conclusion, our work suggests that natural or therapeutic thymic restriction may be beneficial in cancer treatment.

P.B1.02.02

Exploring novel anti-tumor roles of genome-damaging AID/APOBEC3 enzymes in breast cancer

M. Asgharpour*, M. Larijani;*
Immunology and Infectious Diseases Program, Division of BioMedical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St John s, Canada.

Introduction: AID/APOBEC3 cytidine deaminases are genome-editing enzymes that function to boost immunity. It is established that AID/APOBEC3s are an endogenous source of DNA damage leading to initiation and evolution of different cancer types, including breast cancer. We hypothesized that depending on expression levels, AID/APOBEC3s could also have anti-tumor functions.
Methods & Results: We established a first of its kind inducible expression system in which we control expression levels of AID, APBOEC3B and APOBEC3G in breast cancer cells. We verified enzymatic activity of each enzyme and inducible expression of RNA and protein was demonstrated by qRT-PCR, fluorescent microscopy, and flowcytometry. Using MTT, apoptosis and wound-healing migration assays, we observed both tumorigenic and anti-tumor effects depending on different expression levels of the enzymes. The observed effects of AID/APOBEC3s on tumor behavior were in accordance with gene expression levels of key factors involved in cancer cell death and migration as assessed by qRT-PCR.
Conclusion: AID/APOBEC3s play both pro- and anti-tumor roles depending on expression level. This finding represents a paradigm shift as AID/APOBEC3s have thus far been considered strictly as pro-tumor agents. It suggests the possibility of future therapeutic avenues through exploiting these enzymes.
Acknowledgment: This research is supported by funding from CIHR and IDRC. Mahdi Asgharpour is a trainee in the Cancer Research Training Program of BHCRI, with funds provided by TFRI.

P.B1.02.03

Enhanced expression of CCL5 upon the LCMV infection intratumorally leads to the massive NK cells migration to the site of the tumor that further promotes tumor regression

H. A. Bhat[1,2];
[1]*Institute of Immunology, Essen, Germany,* [2]*University Hospital Essen, Instute of Immunology, Uniklinic Esen, Germany.*

In our melanoma model, we used two different human melanoma cells lines Mamel-86A and Mamel-51 we grow both of these cells lines in the NODSCID mice and when the tumor was of measurable size we treated it with lymphocytic choriomeningitis Virus (LCMV) intratumorally. Mamel-86A showed the massive regression where as Mamel-51 turned to be resistant to our therapy. To further, address the question we check the chemokine profile and we also look for interferon upregulating genes and Pro-Apoptotic genes. What we find that many interferon genes are strongly upregulated in the Mamel-86A and in Mamel-51 the expression is very poor likewise, pro-apoptotic genes are also highly expressed in the Mamel-86A compared to the Mamel-51. When we look for the chemokine profile we analysed that CCL5 and CCL10 were strongly upregulated in the Mamel-86A compared to the Mamel-51, But CCL5 showed the highest upregulation than all other chemokines. To further address this question of massive regression upon LCMV infection in Mamel-86A we focused on NK cells whether they might have the role to play in our melanoma model. We were wondering may be some other cells types might be involved for that reason we infect the NSG mice with the Tumor (Mamel-86A) and treated the one group with LCMV to our surprise the tumor growth did not showed any sign of regression even with the LCMV injection intratumorally. we found out upon LCMV injection more and more NK cells migrate to the site of the tumor and lead to regression.

P.B1.02.04

Human recombinant Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) secreted by *Lactococcus lactis* acts synergistically with cytostatics in elimination of human colon cancer cells *in vitro*.

***K. Ciacma**, J. Wieckiewicz, M. Siedlar, J. Baran;*
Jagiellonian University Medical College, Kraków, Poland.

Introduction: One of the leading problems in the current treatment of colon cancer is resistance of the tumor cells to chemotherapy. TRAIL is a natural protein that effectively kills many types of tumor cells and potentially may act synergistically with some chemotherapeutics. However, the biological half-life of TRAIL in mammalian organism is very short, significantly affecting its therapeutic effectiveness. The aim of our study is to investigate, if non-pathogenic *Lactococcus lactis* bacteria can be used as a safe carrier of the TRAIL, enabling both, the control of TRAIL secretion over a period of time and elimination of tumor cells *in vitro*.
Methods: Recombinant plasmid harbouring hsTRAIL-cDNA was constructed and transformed *via* electroporation into *L.lactis* NZ9000 cells. Synthesis and secretion of hsTRAIL was determined in broth supernatants by PCR, ELISA and Western blot. Antitumor activity of hsTRAIL in broth supernatant, used as a single agent and in combination with 5-fluorouracil (5-FU), irinotecan (CPT-11), metformin (MetF), puromycin (Puro) against human colon cancer HCT116 cells was examined *in vitro* by MTS assay. Apoptosis of cancer cells was confirmed by Annexin V binding and flow cytometry analysis. Elimination of HCT116 cells in a co-culture with *L.lactis*(hsTRAIL+) was assessed by MTS assay.
Results: hsTRAIL produced by *L.lactis*(hsTRAIL+) effectively kills HCT116 cells and acts synergistically with cytostatics: 5-FU, CPT-11, MetF and Puro, enhancing elimination of colon cancer cells *in vitro*.
Conclusion: *L.lactis*(hsTRAIL+) bacteria produce biologically active hsTRAIL with potential application for colon cancer immunotherapy.
Acknowledgments: Study supported by National Science Centre (UMO2014/15/B/NZ5/03484) and H2020-MSCA-RISE-2017 (777682, CANCER).

P.B1.02.05

Bovine herpesvirus 4-based vector delivering the full length xCT DNA efficiently protects mice from mammary cancer metastases by targeting cancer stem cells Bovine herpesvirus 4-based vector delivering the full length xCT DNA efficiently protects mice from mammary cancer metastases by targeting cancer stem cells

***L. Conti**[1], G. Donofrio[2], G. Tebaldi[2], S. Lanzardo[1], R. Ruiu[1], E. Bolli[1], A. Ballatore[1], V. Rolih[1], F. Macchi[1], F. Cavallo[1];*
[1]MOLECULAR BIOTECHNOLOGY CENTER, TURIN, Italy, [2]University of Parma, PARMA, Italy.

Despite marked advancements in its treatment, breast cancer is still the second leading cause of cancer death in women aged 20 to 59 years, due to relapses and distal metastases. Breast cancer stem cells (CSCs), are a cellular reservoir for recurrence, metastatic evolution and disease progression, making the development of novel therapeutics that target CSCs, and thereby inhibit metastases, an urgent need. We have previously demonstrated that the cystine-glutamate antiporter xCT (SLC7A11), a protein that was shown to be overexpressed in mammary CSCs and that plays a key role in the maintenance of their redox balance, self-renewal and resistance to chemotherapy, is a potential target for mammary cancer immunotherapy. We developed an anti-xCT viral vaccine that is based on the bovine herpesvirus 4 (BoHV-4) vector, which we have previously showed to be a safe vaccine that can transduce cells in vivo and confer immunogenicity to tumor antigens. We show that the vaccination of BALB/c mice with BoHV-4 expressing xCT (BoHV-4-mxCT), impaired lung metastases induced by syngeneic mammary CSCs both in preventive and therapeutic settings. Vaccination induced T lymphocyte activation and the production of anti-xCT antibodies that can mediate antibody-dependent cell cytotoxicity (ADCC), and directly impair CSC self-renewal and redox balance. Our findings pave the way for the potential future use of BoHV-4 vectors that target xCT in metastatic breast cancer treatment.

P.B1.02.06

High-dimensional profiling of immune subsets in mouse glioblastoma models reveals a tumor-induced 'tolerogenic' microenvironment.

***M. H. W. Crommentuijn**, S. T. Schetters, S. A. Dusoswa, L. J. Kruijssen, Y. van Kooyk;*
VU University Medical Center, Amsterdam, Netherlands.

Tumor cells can manipulate their microenvironment to suppress anti-tumor immunity, which affects patient survival. Modulation of inhibitory immune checkpoints through antibody therapy has shown promising results in several types of cancer, especially of those expressed by T cells. Glioblastoma is the most common, malignant form of primary brain cancer with a very dismal prognosis. As current therapies are insufficient, immune checkpoint inhibition could prove useful. Using advanced multiparameter flow cytometry and t-SNE unsupervised clustering, we performed a high-dimensional subset analysis and assessed co-expression of immune checkpoints and immune checkpoint ligands in two different mouse glioblastoma models. When comparing systemic effects with the brain tumor microenvironment and contralateral hemisphere of the same mouse, we observed a significant increase of CD4 T cells with a highly specialized phenotype, characterized by expression of TIGIT, PD-1, and, to a lesser extent, HVEM. PD-1 expression was also significantly increased on both OVA-antigen-specific CD8 T cells and non-OVA-antigen-specific CD8 T cells. Furthermore, analysis of the myeloid compartment showed massive infiltration of macrophages in the brain tumor microenvironment. Co-expression of immune checkpoint ligands such as PD-L1, CD155 and BTLA was observed on both microglia and infiltrating macrophages. Together, these results suggest a glioblastoma-induced 'tolerogenic' tumor microenvironment, within several different populations of infiltrating immune cells. Upregulation of certain immune checkpoints on lymphoid cells or their ligands on myeloid or tumor cells, could serve as potential targets for combination therapy.

P.B1.02.07

Expansion and characterization of human tumor infiltrating lymphocytes (TILs) in colorectal cancer (CRC)

***M. Español-Rego**[1], R. Cabezón[2], G. Flórez-Grau[3], C. España[1], E. Pineda[4,5], A. Ginés[6], M. Juan[1,2], J. Maurel[4,5], D. Benítez-Ribas[1,7];*
[1]Immunology department, Hospital Clínic de Barcelona, Barcelona, Spain, [2]Institut d'investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, [3]Tumor Immunology, RIMLS, Radboudumc, Nijmegen, Netherlands, [4]Medical Oncology Department, Hospital Clínic de Barcelona, Barcelona, Spain, [5]Translational Genomics and Targeted Therapeutics in Solid Tumors Group, IDIBAPS, University of Barcelona, Barcelona, Spain, [6]Department of Gastroenterology, CIBERehd, Hospital Clínic de Barcelona, Barcelona, Spain, [7]Fundació Clínic, Barcelona, Spain.

Introduction
CRC is one of the most frequent cancer worldwide and less than 40% of patients remain free of progression 12 months after diagnosis. Immunotherapeutic approaches to enhance anti-tumour T cell response have been developed, such us vaccination, immune-checkpoint inhibitors or adoptive cell therapy.
Our aim was to expand and characterize CRC TILs, as a preclinical work useful in tumour immunology
Methods
Eleven primary CRC tumour biopsies were obtained from 9 patients undergoing colonoscopy. Biopsies were obtained from 7 untreated patients and 2 patients before treatment and after first-line progressive disease. Biopsies were processed to obtain a cell suspension by mechanical and enzymatic dissociation. Cells obtained were plated for TIL growth in medium supplemented with IL-2 and anti-CD3-CD28. TILs phenotype was evaluated by flow cytometry: CD3, CD4, CD8, PD1, CTLA4, IFNγ, IL17 and IL10. Data was acquired on FACSCanto II^{BD} and analyzed using BD-FACSDiva software.
Results
TILs were obtained in 10/11 tumour samples. Generally, after 2-weeks of expansion a minimum amount of $4x10^6$ TILs were expanded (mean $35,3x10^6$). On average, 97.3% of TILs were CD3+, with a CD4/CD8 ratio of 9.6. Expression of PD1 and CTLA4 was 1.9% and 0.3% in CD4+ and 1% and 0.3% in CD8+, respectively. 47.8% CD4+ and 71% CD8+ expressed IFNγ, while 21.2% CD4+ and 37.5% CD8+ expressed IL17. No expression of IL10 was detected.
Conclusion
This work shows our capability to obtain TILs and provides new data about the tumoral microenvironment in CRC that could be applied to design new therapies for these patients.

P.B1.02.08

Anti-migratory property of the dual delivery of SN38-Snail siRNA CMD-chitosan nanoparticles on prostate cancer cells

***M. Farzi**[1], A. Afkham[2], S. Sadreddini[2], S. Dolati[3], N. Manafi Afkham[1], M. Yousefi[1];*
[1]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, TABRIZ, Iran, Islamic Republic of, [2]Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, TABRIZ, Iran, Islamic Republic of, [3]Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran, TABRIZ, Iran, Islamic Republic of.

Background: Prostate cancer is the leading cancer diagnosed in men in the US each year. Chitosan nanoparticles have become of great interest for nanomedicine, biomedical engineering and development of new therapeutic drug release systems specially siRNA. Snail and SN38 proteins have moved into the fast lane of development and cancer biology with the epithelial-mesenchymal transition (EMT). In the present study, we aimed to design chitosan/CMD nanoparticles for the efficient encapsulation of the anti-cancer drugs SN38 and Snail-specific siRNA. Methods: Physicochemical characteristics of the synthesized chitosan nanoparticles (140KD) were analyzed using Scanning Electron Microscopy (SEM). Serum & heparin stability and in vitro released assays were done. Anti-migratory property of the dual delivery of SN38-Snail siRNA CMD-chitosan nanoparticles was investigated through quantitative measurement of E-cadherin and Claudin-1 expression level in PC-3 human metastatic prostate cancer cell line. The potential effects of the nanoparticles on migration capability of the prostate cancer cells was also assessed using wound healing assay. Results: Our findings evidently showed 3.12 (±0.62) and 3.02 (±0.28) fold increases in E-cadherin and Claudin-1 mRNA after 24h, respectively.

The upregulation of E-cadherin and Claudin-1 mRNA were continued to 5.6 (±0.91) and 4.42 (±0.61) fold after 48h, respectively. Moreover, co-delivery of SN38and Snail specific siRNA by chitosan nanoparticles resulted in significant inhibition of PC-3 cells migration behavior. Conclusions: In conclusion, In conclusion, our results revealed that dual delivery of ChNPs encapsulating SN38 and Snail-specific siRNA may has a great impact on the treatment of prostate cancer.

P.B1.02.09

Polymer-mediated tumor immunotherapy by in situ activation of antigen presenting cells

J. Hahlbrock[1]*, D. Arnold-Schild*[1]*, J. Stickdorn*[2]*, J. Braun*[3]*, M. Bros*[3]*, S. Grabbe*[3]*, L. Nuhn*[2]*, H. Schild*[1]*;*
[1]Institute for Immunology, Mainz, Germany, [2]Max Planck Institute for Polymer Research, Mainz, Germany, [3]Department of Dermatology, Mainz, Germany.

Immunotherapy has become a promising tool to treat cancer as shown by the use of checkpoint inhibitors. However, dependent on the tumor entity, there are still high recurrence rates and many patients suffer from immune related adverse events. Consequently, there is still high medical need for the development of specific tumor immunotherapies. We were able to design nanogel formulations which are degradable under acidic conditions and can be functionalized by covalent binding of the TLR7-agonist IMDQ and the tumor antigen OVA. Both, the nanoparticle itself and OVA are fluorescently labeled, which allowed biodistribution analyses. Intravenous application revealed an accumulation of the nanoparticles in the spleen with OVA-positive macrophages and elevated B cell numbers. Additionally, we found an uptake of functionalized nanoparticles in BMDCs as well as an enhanced BMDC maturation status. Interestingly, we observed a higher $CD8^+$ T cell response after treatment with nanoparticles which were functionalized through covalent binding of IMDQ and OVA (NP(IMDQ+OVA)) compared to a higher $CD4^+$ T cell response after treatment with nanoparticles to which IMDQ was covalently bound but OVA added in a soluble form (NP(IMDQ)+sOVA). In line with these results, we observed an increased IgG2a antibody production in sera of mice which were immunized with NP(IMDQ)+sOVA and an increased $CD8^+$ T cell response in splenocytes derived from mice treated with NP(IMDQ+OVA). Taken together, these results show promising effects of functionalized nanoparticles which involve $CD4^+$ and $CD8^+$ T cells as well as B cells. Next, therapeutic effects in mice carrying OVA-expressing tumors will be analyzed.

P.B1.02.10

Tumour escape in the microenvironment of penile carcinoma - PD-L1 related parameters predict lymph node metastases and survival

S. Ottenhof[1]*, N. Pocorni*[1]*, R. Djajadiningrat*[1]*, H. Thygesen*[1]*, J. de Jong*[2]*, T. de Gruijl*[3]*, S. Horenblas*[4]*,* ***E. S. Jordanova***[5]*;*
[1]Dutch Cancer Center, Amsterdam, Netherlands, [2]Dutch Cancer Center-AvL, Amsterdam, Netherlands, [3]CCA-VUmc, Amsterdam, Netherlands, [4]Dutch Cancer Center_AvL, Amsterdam, Netherlands, [5]Center for Gynecologic Oncology Amsterdam, Amsterdam, Netherlands.

In the complex interplay between cancer and the hosts immune system, the tumour is threatened by the natural anti-tumour response of cytotoxic T-cells (CTL). However, CTL's are 1) inhibited by regulatory T-cells (Tregs), 2) mislead by aberrant HLA expression by the tumour cells and 3) deactivated by Programmed Death Ligand 1 (PD-L1) on tumour cells or on tumour infiltrating macrophages (TIM). This study aims to gain insight in immunological factors and their prognostic value for lymph node metastases and disease specific survival (DSS) in penile cancer (n-213). HPV-status, different levels of HLA-expression and PD-L1-expression on tumour, stroma and TIM were known from previous studies. Sections were stained for macrophage-marker CD163, CTL-marker CD8, and Treg-marker FoxP3. These parameters were included in multivariable regression models testing the prognostic value for lymph node involvement (LN+ or LN-) and DSS. Multivariable analyses showed three independently prognostic factors for both lymph node status and DSS: 1) PD-L1+ TIM (unfavourable), PD-L1 margin expression (favourable), and a high intra-tumoral CTL/Treg ratio in the presence of PD-L1+ TIM (favourable). This means that PD-L1+ TIM and PD-L1 margin expression were independently predictive in absence of HPV. A PD-L1 expression pattern predominantly at the tumour-stroma margin predicts good prognosis, while the negative predictive value of PD-L1+ TIM appear to be compensated by a high CTL/Treg ratio. These results strengthen the rational for anti-PD-1/PD-L1 immunotherapy in penile carcinoma.

P.B1.02.11

Drug-induced hyperploidy stimulates an antitumor NK cell response mediated by NKG2D and DNAM-1 receptors

S. Lorenzo-Herrero*, C. Sordo-Bahamonde, A. Acebes-Huerta, A. R. Folgueras, L. Huergo-Zapico, S. González, A. López-Soto;*
University of Oviedo, Oviedo, Spain.

Introduction: Formation of polyploid or aneuploid cells is a pathological hallmark of malignant tumors. In addition to cell cycle checkpoints, cancer cell DNA ploidy is subjected to extrinsic controls operated by activation of T-cell mediated immune responses. Whether the innate immune system, and specifically natural killer (NK) cells, have a role in this process has not been deciphered yet.
Materials and Methods: Tumor cell lines were exposed to hyperploidy-inducing agents and surface expression of NK cell ligands was analyzed by flow cytometry. NK cell cytotoxic activity and cytokine production was evaluated after co-culture with treated tumor cells. Pharmacological and genetic approaches were used to study the intracellular mechanism involved in the upregulation of NK cell ligands in hyperploid cancer cells.
Results: Herein, we report that drug-induced polyploidy in cancer cells activates antitumor responses mediated by NK cells. Hyperploidy-inducing agents strongly upregulate the surface expression of NKG2D and DNAM-1 ligands in tumor cells. Further, drug-induced hyperploidy modulates the repertoire of activating receptors and the cytokine profile of NK cells, rendering tumor cells more susceptible to NK cell-mediated lysis. Two stress-related signaling pathways, DNA damage and endoplasmic reticulum stress responses, were involved in the stimulation of MICA, a key NKG2D ligand, in hyperploid cells.
Conclusion: Overall, our findings indicate that, besides the cytotoxic effect on tumor cells, the therapeutic activity of anti-mitotic drugs can also be mediated by the induction of a coordinated antitumor immune response involving NK cells.

P.B1.02.12

TARGETING CD70 FOR THE TREATMENT OF B CELL LYMPHOMA

L. Rohrbeck*, J. Ma, J. Coquet;*
Karolinska Institutet, Stockholm, Sweden.

CD70 is a member of the TNF family that is typically only transiently expressed on several types of immune cells in settings of immune activation. It ligates its receptor CD27, thereby activating various signaling pathways within the target cell. In contrast, CD70 is highly and persistently expressed on a number of malignant cells, particularly on B cell lymphomas. This finding suggests an advantageous role for CD70 in these cells. To date, very few studies have looked into the ability of CD27 to activate CD70 and hence knowledge about the function of CD70 activation in cancers and its downstream signaling pathway(s) is unknown.
The aim of this project is to study the role of CD70 in B cell lymphomas and multiple myeloma and to investigate how cancers benefit from the expression of this protein. So far, we have generated a couple of CD70-deficient cell lines using CRISPR/Cas9 technology. Genome-wide expression arrays will be performed to identify downstream targets of CD70, while knock-out lines will serve as negative controls. Cell proliferation and survival of treated and untreated CD70 knockout cells in combination with various anti-cancer therapeutics will also be assessed by flow cytometry, Finally, we will test the function of CD70-positive and -negative cell lines in mice by monitoring lymphoma growth, drug sensitivity and their impact on the tumor microenvironment. Our preliminary analysis suggests that stimulation of CD70 on cancer cells may promote the immune suppressive cytokine IL-10.

P.B1.02.13

Repurposing antiviral T cells to fight tumors

P. C. Rosato*, M. J. Stolley, S. P. Wijeyesinghe, C. E. Nelson, R. L. Davis, L. S. Manlove, C. A. Pennell, B. R. Blazar, M. A. Geller, A. Grad, V. Vezys, D. Masopust;*
University of Minnesota, Minneapolis, United States.

Overcoming the immunosuppressive tumor microenvironment remains a major impediment to successful cancer immunotherapy. Virus-specific memory T cells are positioned throughout the entire body to sense reinfection and recrudescence. When that same virus is reencountered, these T cells sound an alarm that induces a local immunostimulatory environment that activates and recruits many arms of the immune system. As memory T cells are present in abundance in nearly every tissue and can be triggered by cognate peptide alone, we tested whether we could hijack antiviral CD8+ T cells in tumors to reverse the immunosuppressive tumor microenvironment. We observed that, like healthy tissue, mouse and human tumors are commonly surveyed by virus-specific memory CD8+ T cells. In mouse tumor models, local delivery of adjuvant-free peptide derived from previously encountered viruses recapitulated the sensing and alarm T cell function: recruiting and activating both the innate and adaptive immune system. This viral peptide therapy arrested mouse melanoma tumors and treatment synergized with anti-PD-L1 checkpoint blockade to eliminate measurable tumors and prevented recurrence in 33% of mice. Over half of cured mice rejected subsequent tumor challenges at distant sites, indicating that effective systemic tumor-specific immunity was established. Furthermore, the transcriptional inflammatory response seen in mouse tumors was recapitulated in *ex vivo* organotypic slice cultures of human tumors, supporting the clinical translatability of this therapy. This study demonstrates that natural and existing antiviral immunity can be repurposed to fight tumors without the need for adjuvant, reinfection, or personalized identification of immunogenic tumor neo-antigens.

P.B1.02.14

The combination of magnetic nanoparticles and magnetic fields induces T cell retention both *in vitro* and *in vivo*

L. Sanz-Ortega[1], *J. M. Rojas*[1], *A. Marcos*[2], *Y. Portilla*[1], *J. V. Stein*[2], *D. F. Barber*[1];
[1]*Department of Immunology and Oncology, Centro Nacional de Biotecnología, Madrid, Spain,* [2]*Theodor Kocher Institute, Bern, Switzerland.*

Introduction: A main limitation in cell-based therapies is the dispersion of the administered cells resulting in a small proportion reaching the site of interest. Manipulating cells to target a region could be a strategy to solve this problem. T cells loaded with magnetic nanoparticles (MNPs) could be used for this purpose as long as their functions are not impaired. Here, we evaluate whether MNPs could serve to magnetically guide T cells. **Methods:** Jurkat and murine T cells were used to assess several aspects of T cells after MNP treatment. MNP interactions with T cells together with some important cell functions were evaluated. The *in vitro* and *in vivo* manipulation of MNP-loaded cells with an external magnetic field (EMF) was also analyzed. **Results:** MNPs remain mainly in the cell membrane of T cells. MNPs did not alter cell surface markers expression but slightly reduce the chemotactic response, which can be rescued using EMFs. EMFs can also enhance the *in vitro* retention of MNP-loaded cells in flow conditions. Moreover, we observed an *in vivo* accumulation of T cells in the lymph nodes (LNs) promoted by MNP loading and enhanced by localized EMFs. Finally, MNPs and EMF can reduce the speed of naïve T cells in the LNs. **Conclusions:** This work shows the use of MNPs and EMFs to guide and retain T lymphocytes to certain regions without affecting crucial biological aspects. These studies reveal an interesting approach to promote cell retention that could be implemented in cell-based therapies to improve their efficacy.

P.B1.02.16

Reinforcing dendritic cells for cancer immunotherapy: diverse ways to target antigens to human skin

L. Bellmann[1], *C. H. Tripp*[1], *J. Schulz*[2], *B. del Frari*[1], *P. Milne*[3], *M. Collin*[3], *C. Münz*[4], *C. Rademacher*[2], *N. Romani*[1], ***P. Stoitzner***[1];
[1]*Medical University of Innsbruck, Innsbruck, Austria,* [2]*Max Planck Institute of Colloids and Interfaces, Potsdam, Germany,* [3]*Newcastle University, Newcastle, United Kingdom,* [4]*University of Zürich, Zürich, Switzerland.*

Dendritic cells (DC) are essential for the induction of primary immune responses, and hence preferred targets for immunotherapy of cancer. Skin DC express C-type lectin receptors such as Langerin or DEC-205 for recognition of antigens. Langerin is expressed mainly on Langerhans cells (LC), whereas DEC-205 is expressed by dermal DC and LC. We aim to load skin-resident DC with antibody-antigen fusion proteins directed against these C-type lectin receptors or with antigens encapsulated in liposomes coated with a Langerin ligand. Langerin monoclonal antibody (mAb) injected intradermally into human skin explants was detected exclusively in LC, whereas DEC-205 mAb targeted both dermal DC and LC. A model antigen (EBNA1) fused to DEC-205 mAb elicited EBNA1-specific T cell responses. Liposomes coated with a Langerin ligand showed exclusive binding to LC in cell suspensions obtained from healthy human skin. These liposomes were rapidly incorporated into LC as visualized by confocal microscopy. Furthermore, to test our vaccination approaches in an *in vitro* model, we generated monocyte-derived Langerhans-like cells, which displayed between 50-80% of Langerin expression on the surface and also showed upregulation of CD83 and HLA-DR upon stimulation with a maturation cocktail. In summary, our study will provide a deeper insight into DC-targeted cancer vaccines, their uptake, intracellular trafficking and antigen processing in skin DC. Furthermore, liposomes provide a flexible platform that will allow us to encapsulate antigens to investigate their potential for targeted delivery. Ultimately, this DC-based immunotherapy can be used to increase response rates when used in combination with immune checkpoint inhibitors.

P.B1.02.17

Phenotypic patterns of tumor-infiltrating T and NK cells reflect tumor grading in renal carcinoma

Z. Strizova[1], *P. Taborska*[1], *D. Stakheev*[1], *K. Havlova*[2], *S. Vesely*[2], *J. Bartunkova*[1], *D. Smrz*[1];
[1]*Department of Immunology, 2nd Faculty of Medicine, Charles University in Prague and Motol University, Prague, Czech Republic,* [2]*Department of Urology, 2nd Faculty of Medicine, Charles University in Prague and Motol University, Prague, Czech Republic.*

INTRODUCTION: Adoptive cell immunotherapy (ACI) is a promising approach in the treatment of multiple cancers. However, its efficacy in treatment of renal cell carcinoma (RCC) is low. Low efficacy is caused by a failure to obtain highly potent tumor-reactive lymphocytes after expansion of tumor-infiltrating lymphocytes (TILs). Therefore selective expansion of TIL populations with a potent cytotoxic and migratory activity is needed. In our study we evaluated the localization of cytotoxic/migratory NK, NKT and T cells infiltrating the tumor, peritumoral and adjacent healthy renal tissue. METHODS: 14 patients who underwent radical nephrectomy were included in the study. 42 tissue samples were obtained from the kidney, the tumor and peritumoral tissue, sliced and enzymatically dissociated into single cell suspensions. These cells were then analyzed for the expression of established markers of lymphocyte cytotoxicity - TRAIL and FasL, and a surrogate marker of lymphocyte migratory activity - PECAM-1. The stained cells were analyzed by flow cytometry and the findings correlated with clinical and histopathological data. RESULTS: A tendency to a higher tumor infiltration with PECAM-1$^+$FasL$^+$ T and NK cells was observed in low grade tumors (grade 2) rather than in poorly differentiated high grade tumors (grade 3). In NK cells, this trend was significant (P=0.003). Moreover, PECAM-1$^+$FasL$^+$ NK cells were most frequent in peritumoral tissue. CONCLUSION: The frequency of cytotoxic/migratory NK, NKT and T cells within the tumor and the surrounding milieu is affected by tumor grading. These results may provide important information for development of TIL expansion protocols for ACI of RCC.

P.B1.02.18

Expanded tumor infiltrating lymphocytes upregulate 4-1BB in response to renal cell carcinoma

S. D. van Asten[1,2], *R. de Groot*[3,2], *M. M. van Loenen*[3,2], *A. Bex*[4], *J. de Jong*[4], *D. Amsen*[3,2], *R. M. Spaapen*[1,2], *M. C. Wolkers*[3,2];
[1]*Dept. of Immunopathology, Sanquin Research, Amsterdam, Netherlands,* [2]*Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands,* [3]*Dept. of Hematopoiesis, Sanquin Research, Amsterdam, Netherlands,* [4]*The Netherlands Cancer Institute, Amsterdam, Netherlands.*

The transfer of autologous tumor infiltrating lymphocytes (TILs) is a promising therapy for solid tumors. Patients suffering from renal cell carcinoma (RCC) respond to immunotherapy such as high dose IL-2, albeit with severe toxicities. TIL therapy may therefore be more suitable to treat this tumor type. Previously we successfully expanded tumor reactive TILs from non-small cell lung cancer (NSCLC). Here we cultured TILs from 16 RCC patients. The proliferation rates were lower compared to what we previously found for non-small cell lung cancer (NSCLC) arguing for the presence of suppressive tumor derived factors. T cells of 11 out of 16 patients showed clear upregulation of the activation marker 4-1BB upon exposure to tumor digests and no or lower reactivity to healthy kidney tissue from the same patient.The tumor-specific upregulation of other activation markers (CD40L, CD107a, PD1) was only found in a subset of patients.
Surprisingly, tumor-specific activation was rarely accompanied by the production of IFN-γ, TNF-α or IL-2, while these cytokines were abundantly expressed by expanded NSCLC-derived TILs. This suggests that tumor-reactive 4-1BB positive T cells produce another yet to be defined cytokine profile. To define the tumor microenvironment and its effects on TILs, we are currently correlating the tumor reactivity with a comparative analysis of lymphoid infiltrates to the corresponding healthy kidney tissue. In conclusion, expanded T cells from RCC are tumor reactive as evidenced by 4-1BB upregulation, but the TIL expansion conditions may require further optimization. RCC is thus a conceivable candidate for the application of TIL therapy.

P.B1.02.19

Combined immune stimulation with IL-15 and CD40 results in profound anti-tumour effects in pancreatic cancer

J. R. M. Van Audenaerde[1,2], *B. von Scheidt*[2], *A. Unsworth*[2], *E. Marcq*[1], *A. Oliver*[2], *J. De Waele*[1], *G. Roeyen*[3], *C. Y. Slaney*[2], *P. K. Darcy*[2], *M. Peeters*[1,4], *M. H. Kershaw*[2], *E. L. Smits*[1,5];
[1]*Center For Oncological Research, University of Antwerp, Wilrijk, Belgium,* [2]*Cancer Immunotherapy and Immune Innovation Laboratory, Peter MacCallum Cancer Centre, Melbourne, Australia,* [3]*Dept of Hepatobiliary, Endocrine and Transplantation Surgery, Antwerp University Hospital, Antwerp, Belgium,* [4]*Dept of Oncology and Multidisciplinary Oncological Centre Antwerp, Antwerp University Hospital, Antwerp, Belgium,* [5]*Center for Cell Therapy and Regenerative Medicine, Antwerp University Hospital, Antwerp, Belgium.*

Background: Pancreatic cancer (PC) is the 3rd deadliest cancer worldwide with the lowest 5-year survival of all cancers. Therapeutic improvements have barely been made over the last decade. Within the tumour microenvironment, tackling the stromal shield is needed to overcome treatment resistance. CD40 stimulation has already demonstrated moderate anti-tumour responses in PC, including some anti-stroma effects. We have shown that interleukin (IL)-15 stimulated NK cells are capable of tackling both tumour as well as the surrounding desmoplastic stroma. Therefore, we explored a novel combination immunotherapy consisting of an agonistic anti-CD40 monoclonal antibody and IL-15 in two mouse models of PC.
Methods: C57BL/6 mice bearing subcutaneous Panc02 or KPC tumours were treated over a two-week period with IL-15 and/or anti-CD40. Tumour kinetics and survival were monitored. Experiments depleting different immune cell populations were performed. Re-challenge experiments were executed to check immune memory induction. Tumour infiltrating lymphocytes are being characterised using flow cytometry and immunohistochemistry.
Results: Combination treatment of IL-15 and anti-CD40 caused a distinct reduction of tumour growth rates in comparison with single agent treatments. Mice receiving the combination treatment showed significantly increased survival, with 60-80% of the mice being completely tumour free. Depletion studies revealed both CD8+ T cells and NK cells are mechanistically involved in the anti-tumour effect of this novel treatment. Re-challenge experiments also showed that immune memory was induced. Flow cytometry experiments and immunohistochemistry experiments are being performed to provide more details on the phenotype of the tumour infiltrating lymphocytes and their spatial distribution within the tumour.

P.B1.02.20

Induction of immunogenic cell death by innovative antitumoral platinum (II) compounds

M. Wantz[1], *N. Chekkat*[1], *M. Bouché*[2], *G. Dahm*[2], *B. Frisch*[1], *S. Bellemin-Laponnaz*[2], *S. Fournel*[1];
[1]*Faculty of Pharmacy, Illkirch Cédex, France,* [2]*IPCMS, Strasbourg Cédex 2, France.*

Some cancer treatments like chemotherapeutic agents (anthracyclines, platinum derivatives,…) are able to activate the antitumor immune response by inducing a particular cell death: the immunogenic cell death (ICD). This process is characterized by the exposition of the endoplasmic reticulum chaperone calreticulin (CRT) at the cell surface as well as the release of ATP and non-histone chromatin-binding protein high mobility group box 1 (HMGB1) which serve as immunostimulatory damage-associated molecular patterns (DAMPs) and increase the antitumor immune response. We focused on *N*-heterocyclic carbene platinum complexes associated with polyethyleneimine, a transfection agent, to create multivalent cationic platinum compounds (NHC-Pt(II)-PEI) that induce apoptosis *in vitro* and *in vivo* in xenograft immunodeficient mouse model[1].
To evaluate the potential implication of the immune response on the NHC-Pt(II)-PEI *in vivo* effect, immunocompetent mice bearing tumors were treated with platinum particles and the results revealed an antitumor effect of our conjugates, in the same range than the clinical used platinum drug oxaliplatin, but with less side effects. We evaluated if NHC-Pt(II)-PEI were able to induce ICD. First results showed expression of CRT upon NHC-Pt(II)-PEI treatment. We are then evaluating if their association with immune danger signals could enhance this effect.
Altogether our results reveal the possibility of creating Pt(II) derivatives that can be used as chemotherapeutic agents by killing tumor cells and as immunotherapeutic agents by triggering the antitumor immune response.
1 Chekkat et al. Bioconjugate Chem 2016, 27, 1942-1948

P.B1.03 Tumor vaccination principles and Immunotherapy - Part 3

P.B1.03.01

Molecular mechanism for M1 bias of ABCG1-deficient macrophages

M. Altunay[1], *S. Gunalp*[1], *D. Unuvar Purcu*[2], *R. Ozbilgic*[1], *Z. Ayyildiz*[1], *F. Hapil*[3], *Z. Tavsan*[4], *G. Wingender*[1,4], *D. Sag*[4,5];
[1]*Izmir International Biomedicine and Genome Institute, Dokuz Eylul University, Izmir, Turkey,* [2]*Department of Molecular Medicine, Health Sciences Institute, Dokuz Eylul University, Izmir, Turkey,* [3]*Izmir Biomedicine and Genome Center, Izmir, Turkey,* [4]*Izmir Biomedicine and Genome Center, Izmir, Turkey,* [5]*Department of Medical Biology, School of Medicine, Dokuz Eylul University, Izmir, Turkey.*

Macrophages that are major players of tumor immunity, are divided into two subgroups as M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages. In general, M1 macrophages are potent tumor-fighting cells, whereas M2 macrophages display protumoral functions. ATP-binding Cassette Transporter G1 (ABCG1) promotes cholesterol efflux from cells and regulates intracellular cholesterol homeostasis. We have recently shown that in the absence of ABCG1, macrophages shift from a tumor-promoting M2 phenotype to a tumor-fighting M1 phenotype within the tumor and suppress bladder cancer growth in vivo. The molecular mechanism through which ABCG1-deficiency shifts macrophages to an M1 phenotype is not known. AMP-activated protein kinase is a master regulator of energy metabolism. In macrophages, AMPK also regulates M1/M2 polarization and activation of AMPK promotes macrophage polarization to an M2 phenotype. Herein, we show that Abcg1-/- bone marrow-derived macrophages display reduced levels of AMPK activation at baseline and also after stimulation with LPS/IFNγ (M1 stimulus) or IL4 (M2 stimulus). Moreover, while Abcg1-/- macrophages stimulated with LPS/IFNγ have increased TNFα production compared to WT macrophages, after treatment with the AMPK activator AICAR, the TNFα production of Abcg1-/- and WT macrophages were comparable. Our data suggest that the M1 bias of Abcg1-/- macrophages is mediated through AMPK signaling pathway. These findings not only deepen our mechanistic understanding of the M1/M2 switch in macrophages, but have the potential to open up new immunotherapeutic approaches for the treatment of cancer.
L'Oreal UNESCO For Women In Science International Rising Talent Grant (to D.S); Dokuz Eylul University, Research Foundation Grant (2017.KB.SAG.011, to D.S)

P.B1.03.02

Adoptive cell transfer following personalized cancer vaccination elicits newly detectable neoantigen-specific T-cell responses in ovarian cancer patients

V. Bianchi[1], *S. Bobisse*[1], *B. J. Stevenson*[2], *L. Kandalaft*[1], *A. Harari*[1], *G. Coukos*[1];
[1]*Ludwig Institute for Cancer Research, Lausanne, Switzerland,* [2]*Swiss Institute of Bioinformatics, Lausanne, Switzerland.*

Private tumor neo-antigens derived from non-synonymous somatic point mutations can be immunogenic and are becoming highly attractive targets for tailored mutanome-based immunotherapies. A pilot study conducted by Kandalaft and colleagues, has shown that a personalized vaccination strategy in advanced ovarian cancer patients is feasible and safe, and induces a broad antitumor response including T-cell reactivities against private mutated neo-epitopes (Kandalaft et al., 2013; Tanyi et al. 2018). Patients who developed antitumor T-cell responses following vaccination but failed to achieve a complete remission (n=12), were enrolled in a second part of the trial involving lympho-depletion and adoptive cell transfer (ACT) of autologous vaccine-primed *ex vivo* co-stimulated T-cells (Kandalaft et al., 2013). To investigate whether ACT provided a boost to the cancer vaccine treatment, we performed a longitudinal screening of neo-epitope specific T-cells in the peripheral blood of vaccinated patients who received T-cell infusion. Of interest several (n=6) $CD8^+$ T-cell reactivities to private nonsynonymous mutations (neoepitopes) were newly detected only upon ACT in the blood of 4 out of 11 patients. Furthermore, increased progression-free survival was associated with the *de novo* detection of neoepitope-specific T-cell responses after ACT. We are now in the process of further dissecting the frequency of neoantigen-specific T-cell populations in the T-cell infusion products of patients undergoing ACT. Tracking the origin, expansion and persistence of neo-antigen specific T-cell clonotypes upon infusion, will help elucidate whether ACT of *ex vivo* co-stimulated T-cells following personalized vaccination is an effective way to further mobilize T-cell reactivities against the patient's mutanome.

P.B1.03.03

Interferons synergize with either TLRs or CD40-induced signaling to efficiently render macrophages tumoricidal *in vitro*

P. F. Christopoulos[1], *A. Lunde*[1], *E. Müller*[1], *T. A. Theodossiou*[2], *I. Øynebråten*[1], *A. Corthay*[1];
[1]*Tumor Immunology Lab, Department of Pathology, Rikshospitalet, Oslo University Hospital, Oslo, Norway,* [2]*Department of Radiation Biology, Institute for Cancer Research, Radium Hospital, Oslo University Hospital, Oslo, Norway.*

Introduction: TAMs represent a main component of the tumor-infiltrating immune cells and therefore re/polarization into their anti-tumor M1 phenotype has raised great interest in cancer immunotherapy. IFNγ and/or LPS have been described as the typical inducers of the classical M1 activation, however less is known about the effect of other molecules or TLR ligands on macrophages activation.
Methods: LPS, Pam3CSK4, IFNγ, IFNβ and sCD40L were used alone or in combinations to activate mouse BMDM. Growth inhibition and/or apoptosis of LLC and MOPC315 cells were investigated using co-culture *in vitro* assays. Production of NO and cytokines, as well as, other phenotypic properties of polarized BMDM were also analyzed.
Results: We found that IFNγ induced growth inhibition and apoptosis of cancer cells only when it was used in combination with TLR agonists or sCD40L. Similarly, IFNβ also synergized with TLR-ligands for induction of cancer cell growth inhibition. In addition, combinational treatments synergistically upregulated NO, as well as TNFα and IL-12 production in BMDM, whereas IL-10 secretion was suppressed. Furthermore, activated BMDM upregulate CD38, CD40, CD80/86, MHCI/II and PD-L1 in different expression patterns depending on the applied stimuli. The mitochondrial respiration was suppressed upon activation and to a greatest extent following combinational treatments. Finally, apoptosis of macrophages was not greatly affected by activation.
Conclusions: Herein we have shown that TLR agonists, sCD40L and/or interferons promote distinct functional and phenotypic properties in so-called M1 macrophages. We conclude that activation of more than one signaling pathway is required to efficiently induce macrophage tumoricidal *in vitro*.

P.B1.03.04

Astragaloside II Exerted Anti-Tumor Immunological Effect through Regulating CD45

w. Chunping;
Yunnan University of Traditional Chinese Medicine, Kunming, China.

object: This paper was designed to assess the anti-tumor immunological effect and reveal the molecular mechanism on Astragaloside II exhibit the anti-tumor immunological effect via regulating regulating CD45 .
Method: The H22 tumor-bearing mice was established . Mice were divided into three groups including, Model group ,Astragaloside II group and Astragaloside II+Anti-CD45 Ab group.After 10 days of intragastric administration, the tumor tissue were isolated to assess the anti-tumor immunological effect of Astragaloside II, The lymphocyte cells proliferation from H22 tumor-bearing mice was detected by MTT method. The mRNA expression of Th1 cytokine,Th2 cytokine and transcript factor T-bet were examined by q-PCR analysis.Surface marker,Treg and Th1 intracellular cytokines(IFN-γ) were detected by flow cytometry.

Result: The tumor weight,tumor diameter and tumor volume in Astragaloside II group are more small than model group($P < 0.05$). Astragaloside II treatment significantly enhance the lymphocyte proliferation activity from H22 tumor-bearing mice in response to ConA,increase the proportion of $CD44^+CD62L^-$ effector T cells, up-regulated the transcriptional expression in Th1 and Th2,including IFN-γ,IL-2,IL-4 and T-bet.Meanwhile,Astragaloside II treatment also markedly increase IFN-γ expression in CD4+T cells and down-regulated T regulatory cell expression.Furthermore,anti-mouse CD45 Ab treatment intensely blocked the anti-tumor immunological effect and lymphocyte proliferation activity which induced by Astragaloside.Compared with Astragaloside II group,IL-2,IFN-γ, IL-4 and T-bet mRNA expression is decreased in Astragaloside II+Anti-CD45 Ab group.Anti-mouse CD45 Ab treatment intensely down-regulated IFN-γ expression in CD4+T cells without significantly influencing Treg cells and $CD44^+CD62L^-$ effector T cells.
Conclusion: we hypothesis that activating CD45 protein tyrosine phosphatase may be involved in anti-tumor immunological effect of Astragaloside II.

P.B1.03.05
Targeting human skin DC using melanoma specific multivalent glyco-nanomers to enhance anti-tumor immune responses

***S. Duinkerken**, S. T. Schetters, H. Kalay, M. Ambrosini, J. J. Garcia-Vallejo, Y. van Kooyk;*
VUmc, Amsterdam, Netherlands.

Human skin dendritic cell (DC) subsets are actively explored for use in anti-cancer vaccination strategies because of their easy accessibility. Targeting epidermal Langerhans cells (LCs) or dermal $CD1a^+$, $CD14^+$, and $CD141^+$ DCs individually has shown enhanced (cross-) presentation of tumor-associated antigens (TAA), inducing tumor specific $CD4^+$ and $CD8^+$ T cells, yet not enough for effective cancer regression in humans. We hypothesize, that simultaneous targeting of multiple DC subsets might give superior responses *in vivo*. We designed multivalent glyco-nanomers which showed increased $CD8^+$ T cell responses compared to single peptides *in vivo*, showing the potential for use of multivalent CLR targeting moieties to induce anti-tumor T cell responses. To verify targeting capabilities of these nanomers specifically to human skin DCs a human skin explant model was used. Multivalent glyco-nanomers containing gp100 MHCI and II epitopes were coupled to the common DC-SIGN/Langerin ligand Le^y, thereby targeting both LCs and dermal DCs (dDCs). We show increased uptake by LCs, $CD14^+$ and $CD1a^+$ dDCs *in situ* which, surprisingly, was not mirrored with increased *in vitro* $CD8^+$ T cell activation. To ensure proper T cell activation we combined the multivalent nanomers with the PRR agonists MPLA (TLR4) and MDP (NOD2). We show an altered skin environmental milieu when MPLA and MDP-nanomers are injected *in situ* in human skin and enhanced *in vivo* $CD8^+$ T cell responses in B6 mice. In conclusion, we designed tumor specific multivalent glyco-nanomers which can target both CLR and PRR on multiple human skin DC to induce anti-tumor immune responses.

P.B1.03.06
Reduced DNAM expression and impaired function of cytokine-secreting and cytotoxic NK cells in tumor draining lymph nodes in non small cell lung cancer

*E. Cetin Aktas[1], A. Turna[2], **F. Esen**[3,1], A. Engin[1], M. Agkoc[4], G. Deniz[1];*
[1]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Istanbul, Turkey, [2]Istanbul University, Cerrahpasa Medical School, Department of Thoracic Surgery, Istanbul., Turkey, [3]Istanbul Medeniyet University School of Medicine, Department of Ophthalmology, Istanbul, Turkey, [4]Istanbul University, Cerrahpasa Medical School, Istanbul, Turkey.

Natural killer (NK) cells have important functions in antitumoral immunity and have been shown to have impaired functions in cancer patients. The aim of this study was to evaluate phenotyping of NK cells in tumor draining lymph nodes of non-small cell lung cancer (NSCLC).
Five NSCLC lung cancer patients undergoing preoperative mediastinal staging by video-assisted mediastinoscopic lymphadenectomy were included in this study. Peripheral blood mononuclear cells were isolated from tumor draining lymph nodes and peripheral blood. Activator and inhibitory receptors, immune checkpoint molecule expressions were analyzed in NK cell subsets. Cytotoxic capacity and cytokine secretion of NK cells were also analyzed.
The ratio of cytotoxic $CD16^{bright}CD56^{dim}$ (p=0.008) and $CD16^{bright}CD56^-$ NK cells (p=0.03) were significantly diminished in lymph nodes, while there was no difference for cytokine-secreting NK cells (p=0.22). IL-10 secreting NK cells were significantly increased (p=0.02), while IFN-γ (p=0.03) and TNF-α (p=0.03) secreting $CD16^-CD56^{dim/bright}$ cells were significantly decreased in lymph nodes, representing a regulatory phenotype. Percentages of $CD16^{bright}CD69^+$ cells (p=0.047) and granzyme expression of $CD16^{bright}$ cells in unstimulated and K562 stimulated conditions (p=0.016 and p=0.008) were significantly lower in lymph nodes, while there was no statistically significant difference for CD107a degranulation (p=0.20 and p=0.88). DNAM expression in the lymph nodes were significantly lower in both groups (p=0.008).
Tumor responding immune cells reduced DNAM expression of both cytotoxic and cytokine secreting NK cells, that might lead regulatory or suppressive phenotype of these cells to impair antitumoral immune responses.

P.B1.03.07
Exosome based immunotherapy to induce antigen-specific humoral and cellular immunity and mediate long-term memory in vivo

***X. Hu**, G. Gucluler, R. Veerman, P. Larssen, S. Gabrielsson;*
Karolinska Institutet, Solna, Sweden.

Exosomes are candidates for cancer immunotherapy due to their capacity to stimulate tumor-specific activity *in vivo*. However, clinical trials using peptide-loaded autologous exosomes have so far only showed moderate T cell responses, suggesting a need for optimization of exosome-induced immunity in humans. We previously demonstrated that induction of antigen-specific $CD8^+$ T cells and anti-tumor responses to whole antigen were independent of major histocompatibility complex (MHC) class I on exosomes. Here, we further investigated humoral and cellular immunity induced by syngeneic and allogeneic exosomes. Both exosomes can enhance antigen-specific $CD8^+$ T cell, follicular helper T cell (Tfh) and antigen-specific antibody responses. Exosome-injected mice demonstrated antigen-specific memory after 4 months. To be noted, mice receiving double allogeneic exosome injections showed highest antibody avidity. Reduced B16mOVA melanoma tumor growth was shown in all exosome-injected groups. Our findings support the application of allogeneic exosomes for therapeutic use in clinical immunotherapy studies.
This work was supported by grants from the Swedish Research Council Medicine, The Swedish Cancer Foundation, The Cancer Research Foundations of Radiumhemmet, The Swedish Heart-Lung Foundation, Centre for Allergy Research Karolinska Institutet, and the Karolinska Institutet.

P.B1.03.08
Ectopically expressed membrane-bound form of IL-9 exerts immune-stimulatory effect on CT26 colon carcinoma cells

*V. Do Thi, **Y. Kim**;*
Department of Biochemistry, College of Natural Sciences, Chungnam National University, Daejeon, Korea, Republic of.

IL-9 is a known T cell growth factor with pleiotropic immunological functions, especially in parasite infection and colitis. However, its role in tumor growth is controversial. In this study, we generated tumor clones expressing the membrane-bound form of IL-9 (MB-IL-9) and investigated their influences on immune system. MB-IL-9 tumor clones showed reduced tumorigenicity but shortened survival accompanied with severe body weight loss in mice. MB-IL-9 expression on tumor cells had no effect on cell proliferation or major histocompatibility complex class I expression in vitro. MB-IL-9 tumor clones were effective in amplifying CD4+ and CD8+ T cells and increasing cytotoxic activity against CT26 cells in vivo. We also observed a prominent reduction in body weights and survival period of mice injected intraperitoneally with MB-IL-9 clones compared with control groups. Ratios of IL-17 to interferon (IFN)-γ in serum level and tumor mass were higher in mice implanted with MB-IL-9 tumor clones than those observed in mice implanted with control cells. These results indicate that the ectopic expression of the MB-IL-9 on tumor cells exerts an immune-stimulatory effect with toxicity. To exploit its benefits as a tumor vaccine, a strategy to control the toxicity of MB-IL-9 tumor clones should be developed.

P.B1.03.10
Immunomonitoring of triple negative breast cancer patients undergoing neoadjuvant therapy (GBG89, Geparnuevo trial)

***C. Massa**[1], A. Mueller[1], A. Schneeweiss[2], C. Hanusch[3], J. Huober[4], M. Untch[5], T. Karn[6], P. Fasching[7], F. Marmé[2], N. Burchardi[8], C. Denkert[9], S. Loibl[8], B. Seliger[1];*
[1]Martin Luther University, Halle (Saale), Germany, [2]Nationales Centrum für Tumorerkrankungen, Heidelberg, Germany, [3]Rotkreuzklinikum Frauenklinik, München, Germany, [4]University of Ulm, Ulm, Germany, [5]HELIOS Klinikum Berlin Buch, Berlin, Germany, [6]University of Frankfurt, Frankfurt, Germany, [7]University Hospital Erlangen, Erlangen, Germany, [8]GBG German Breast Group, Neu-Isenburg, Germany, [9]Charité University Hospital, Berlin, Germany.

The Geparnuevo trial is a randomized, double-blind, multi-center phase II trial of neoadjuvant therapy in patients with early-stage triple negative breast cancer (TNBC) investigating the role of durvalumab, an anti-PD-L1 inhibitor in addition to standard chemotherapy with nab-Paclitaxel followed by Epirubicin plus Cyclophosphamid. In order to determine possible predictive and / or prognostic biomarkers, blood samples were taken before and during the different treatment phases and evaluated by multicolor flow cytometry. Evaluation of the absolute cell count in the whole blood highlighted a mixed behavior of the total leukocytes, whereas there was a statistically significant reduction in the lymphocytes, particularly during the last phase of the treatment. Further dissection into the different immune populations highlighted an almost complete loss of B cells that in some patients was also accompanied by a reduction of NK cells, mostly regarding the $CD16^+$ subset. However, the loss of $CD4^+$ and $CD8^+$ T cells has been less pronounced resulting in an overall enhancement of their percentages within the total lymphocytes. The different populations have also been evaluated for the expression of activation and exhaustion markers, whose behavior will be more deeply evaluated when the clinical outcome and the treatment received by the various patients will be made available. We expect that with such analysis possible biomarkers for the treatment of TNBC patients will be identified thus leading to better patient selection for tumor chemo/immuno combination therapy. The trial and this translational research project was funded by AZ and Celgene, Germany, respectively.

P.B1.03.11

Flagellin increases death receptor-mediated cell death in a RIP1-dependent manner

T. Molnar[1], *D. Hancz*[2], *A. Szabo*[1], *Z. Varga*[1], *A. Hancz*[2], *A. Gregus*[1], *A. Hueber*[3], *E. Rajnavölgyi*[1,4], *G. Koncz*[1,4];
[1]*Department of Immunology, University of Debrecen, Debrecen, Hungary,* [2]*Immunology Department, University Eötvös Lorand, Budapest, Hungary,* [3]*Institut de Biologie Valrose, CNRS UMR 7277, INSERM UMR, Université de Nice, Nice, France,* [4]*Department of Bioengineering, Sapientia Hungarian University of Transylvania, Cluj-Napoca, Romania.*

Efficient adjuvants have the potential to trigger both innate and adaptive immune responses simultaneously. Flagellin is a unique pathogen-derived protein, which is recognized by pattern recognition receptors (PRRs) as well as by B-cell and T-cell receptors thus providing an important link between innate and adaptive immunity.
Here, we sought to investigate the potential modulatory effects of flagellin exerted on various cell death processes known to play detrimental roles in regulating the final outcome of various types of immune responses. We proved that the pre-treatment of Jurkat T-cells with flagellin is able to increase the degree of cell death provoked by FAS, TRAIL or TNF-α and concomitantly increases the cytotoxic potential of phytohemagglutinin activated T-lymphocytes. In contrast to these flagellin-mediated effects exerted on the death receptor-induced signalling events, the mitochondrial apoptotic pathway remained unaffected. Furthermore, the cell culture supernatant of wild type *Salmonella enteritidis* bacteria, but not their flagellin deficient variant were able to enhance the Fas-induced cell death. To define the molecular mechanisms mediated by flagellin we were able to detect the upregulation of RIP1-dependent signalling events.
These findings demonstrate that the cooperative action of pattern recognition and the different death receptors are able to initiate the cell death process towards the mobilization of RIP-dependent cell death modalities. This finding highlights the capability of flagellin to act as a potential adjuvant relevant for tumor immunotherapy.
The work is supported by NKFIH-K-125224 and by the GINOP-2.3.2-15-2016-00050 project. The project is co-financed by the European Union and the European Regional Development Fund.

P.B1.03.12

Cytokine nitration boosts myeloid suppressor cell commitment and functions in tumors

A. Agnellini[1], *B. Cali*[1], *A. Predonzani*[2], *G. Toffolo*[2], *G. Arrigoni*[1], *F. Zonta*[3], *L. Albertoni*[4], *C. Mescoli*[4], *I. Marigo*[5], ***B. Molon***[1];
[1]*Department of Biomedical Sciences, University of Padova, Padova, Italy,* [2]*Venetian Institute of Molecular Medicine VIMM, Padova, Italy,* [3]*ShanghaiTech University · Shanghai Institute for Advanced Immunochemical Studies, Shanghai, China,* [4]*Department of Medicine, DIMED, Surgical Pathology and Cytopathology Unit, University of Padua, Padova, Italy,* [5]*Istituto Oncologico Veneto, IOV-IRCCS, Padova, Italy.*

The recruitment of defined immune cell subsets within the locoregional tumor environment significantly influence cancer fate. A strong lymphocytic infiltration associate with good clinical outcome in different human tumors. On the contrary, high frequency of myeloid cells is tightly connected with tumor promotion, metastasis and poor prognosis. Despite the relative profusion of each cell type, the predominant cytokine and chemokine milieu within the tumor microenvironment importantly tips the balance in favor of either anti-tumor immunity or tumor-induced immunosuppression. Notably, the overload of inflammatory reactive species is a common feature of the majority of chronic inflammatory diseases including cancer. Reactive Nitrogen Species (RNS) influence homeostatic properties of several proteins at post-translational level. Indeed, the post-translation modifications (PTM) of proteins represent an important level of regulation that must be deeply investigated in cancer. Our group showed that PTM alter the recruitment of distinct immune subsets within tumor primary lesion thus affecting the efficacy of cancer therapy. We originally focused our analysis on CCL2, though other cytokines and chemokines should be target of such modifications and contribute to shape the immune response. One of the possible candidates is the granulocyte-macrophage colony-stimulating factor (GM-CSF), which is an important regulator of inflammation. Its prominent role as immunomodulatory cytokine has been increasingly considered from different studies linking its deregulation to chronic inflammatory diseases and cancer. Our data indicated that RNS impact on the molecular dynamics and functions of this key cytokine by altering the immune landscape in tumor-bearing hosts.

P.B1.03.13

Direct and indirect effects of various cytokine pretreatment of human peripheral blood mononuclear cells on the proliferation of cervical cancer cells in coculture

M. G. Mujtaba*, T. R. Simpson;*
Florida Gulf Coast University, Fort Myers, United States.

Immunotherapies are increasingly being developed to target certain tumors and cancers. Here, we examine the direct and indirect effects of various cytokine pretreatment of human peripheral blood mononuclear cells (HPBMC) on the proliferation of cervical cancer cells. HPBMC were pretreated with various cytokines, such as IL-12, IL-15, IL-18, and IFNγ, either alone or in combination for 48 hours, after which cells were washed. Washed cells were then either incubated directly with HeLa cells or alone for another 48 hours and their supernatants collected and incubated with HeLa cells. After 48 hours of coculture of HeLa cells with either HPBMC or supernatants, cellular proliferation was measured using the WST-1 proliferation assay kit and absorbance was read at 495nm on a microplate reader. Results show that HeLa cells inhibition required direct contact by HPBMC when cytokine pretreatments involved IL-12 and IL-15. Direct coculture inhibition of proliferation was approximately 54% for IL-12 and 62% for IL-15 pretreatments. On the contrary, IL-18 pre-treated HPBMC cell supernatants had a greater effect on HeLa cell inhibition (40%) than HPBMC directly cocultured with HeLa cells (20%). Both IFNγ pretreated HPBMC (33% inhibition) and their resultant supernatants (23% inhibition) had inhibitory effects on HeLa cell proliferation. At optimal inhibitory concentrations, above cytokine combination pretreatments of HPBMC had no additive or synergistic inhibition of HeLa cell proliferation in the coculture assay. Thus, data from this study increases our understanding of the tumor microenvironment and the effect of certain cytokines on peripheral blood mononuclear cell activity on cancer cells.

P.B1.03.15

Porcine Circovirus Type 2 ORF3 protein induces apoptoses in melanoma cells

M. Teras[1], *A. Rump*[2], *V. Paalme*[2], ***S. Rüütel Boudinot***[2];
[1]*North Estonia Medical Centre, Oncology, Estonia,* [2]*Tallinn University of Technology, Department of Chemistry and Biotechnology, Estonia.*

Background. The current treatment of malignant melanoma is limited by the lack of effective therapeutic approaches, and alternative treatments are needed. Proliferative diseases such as melanoma and other cancers may be treatable by virally-encoded apoptotic proteins that are targeted to rapidly multiplying cells. **Methods.** In the current study, the Porcine circovirus type 2 (PCV2), proapoptotic protein ORF3 was expressed in mouse and human cancer cell lines, and its apoptotic activity assessed. **Results**. Quantitative assessment of the apoptotic cells by flow cytometry showed that apoptotic cell death was significantly increased in ORF3-expressing malignant cells, compared with ORF3 non-expressing cells. Our data show that PCV2 ORF3 induces apoptosis likely in a caspase-independent manner. ORF3 expression causes an increase in abnormal mitosis in B16F10 melanoma cells by interacting with centrosomes and thereby disrupting formation of the mitotic spindle. In addition, we show that ORF3 of PCV2 also exhibits significant anti-tumor effects *in vivo*. Although the expression of regulator of G protein (RGS)-16 by recipient mice inhibited the developement of grafted melanoma *in vivo*, it was not required for the antitumoral activity of ORF3. **Conclusion.** PCV2 ORF3 causes non-bipolar mitosis in rapidly dividing cells and increases the apoptosis of cancer cells. Apoptin might therefore be considered to develop future antitumoral strategies.

P.B1.03.16

Cortisol determines the capability of human acute myeloid leukaemia cells to escape immune surveillance via upregulation of latrophilin expression on genomic level

S. S. Sakhnevych[1], *I. M. Yasinska*[1], *A. M. Bratt*[1], *O. Benlaouer*[1], *W. Fiedler*[2], *Y. Ushkaryov*[1], *V. Sumbayev*[1];
[1]*Medway School of Pharmacy, Chatham Maritime, United Kingdom,* [2]*University Hospital Hamburg-Eppendorf, Hamburg, Germany.*

Introduction. Acute myeloid leukaemia (AML) is a blood and bone marrow cancer, which rapidly develops into a systemic malignancy due to capability of cancer cells to disable anti-cancer immunity. One of the biochemical mechanisms lying in the core of this process is highly upregulated secretion of galectin-9, a tandem protein, which triggers biochemical inactivation of natural killer (NK) cells and killing of cytotoxic T cells. Galectin-9 secretion is mediated by latrophilin 1 (LPHN1), a G-coupled receptor expressed by AML cells but not healthy leukocytes. However, the biochemical machinery which controls these events remains unclear and thus was the aim of this study. **Methodology.** Primary human AML cells, healthy leukocytes and blood plasma from respective donors as well as THP-1 human AML cell line were used to conduct the work. Western blot analysis, ELISA and qRT-PCR were employed as main research instrumental. **Results and Discussion.** Cortisol has significantly upregulated LPHN1 expression in AML cells, but not in primary healthy leukocytes. Importantly, cortisol levels were highly upregulated in blood plasma of AML patients compared to healthy donors. Natural LPHN1 ligand, FLRT3 protein present in human blood plasma in its soluble form was found to facilitate galectin-9 exocytosis in AML cells. **Conclusion.** Our results suggest, that human steroid hormone cortisol normally responsible for regulation of metabolism is used by malignant AML cells to gain capability to disable anti-cancer immunity. In blood plasma of AML patients glucose levels are decreased, which induces biochemical triggering of upregulated cortisol production by hypothalamus and pituitary gland.

P.B1.03.17

Combination therapies to improve DC-based treatment of melanoma

A. Seretis[1], *G. Cappellano*[1], *L. Bellmann*[1], *J. Schachtl-Rieß*[1], *N. Prokopi*[1], *D. Ortner-Tobider*[1], *K. Komenda*[1], *C. H Tripp*[1], *C. E Brinckerhoff*[2], *J. Lühr*[3], *C. Lehmann*[3], *D. Dudziak*[3], *P. Stoitzner*[1];
[1]*Department of Dermatology, Venereology and Allergology, Medical University of Innsbruck, Innsbruck, Austria,* [2]*Departments of Medicine and Biochemistry, Geisel School of Medicine at Dartmouth, Norris Cotton Cancer Center, Lebanon, New Hampshire, United States,* [3]*Department of Dermatology, Laboratory of Dendritic Cell Biology, Friedrich-Alexander University Erlangen-Nürnberg (FAU), University Hospital Erlangen, Erlangen, Germany.*

Dendritic cells (DC) are the most potent antigen-presenting cells of the immune system inducing $CD4^+$ and $CD8^+$ T cell responses. However, the tumor microenvironment actively suppresses anti-tumor immunity. The aim of this project is to develop combination therapies, allowing control of melanoma growth. For this purpose, tumor antigens will be delivered directly to DC by antibody-mediated uptake. These DC-vaccines will be combined with tumor-targeted therapy or immune checkpoint inhibitors to further boost anti-tumor immune responses. For the generation of DC-vaccines, the expression of three melanoma-associated antigens (gp100, trp-2 and MAGE-A2) were examined by RT-PCR in two BRAF mutant melanoma models: the transplantable D4M and the inducible Tyr::CreER;$Braf^{CA}$;$Pten^{lox/lox}$. In the D4M model, we observed low levels of expression which increased under treatment with a BRAF inhibitor-containing diet, while the inducible Tyr::CreER;$Braf^{CA}$;$Pten^{lox/lox}$ model showed high expression levels in tumors compared to healthy skin. We are currently cloning these melanoma antigens into the skin DC targeting antibody, anti-DEC-205. These constructs will be used to deliver the antigens to skin DC and combined with the BRAF inhibitor. So far, we observed that the transplantable D4M melanoma model is very sensitive to BRAF inhibition and develops resistance after prolonged exposure. Moreover, we found that the BRAF inhibitor affects the phenotype and composition of immune cell infiltrate, in D4M tumors. Our ultimate goal is to improve anti-tumor immunity and prevent development of resistance to targeted therapy by combining it with DC-targeting vaccines.

P.B1.03.18

The Tim-3-galectin-9 secretory pathway determines the capability of human acute myeloid leukaemia (AML) cells to escape host immune surveillance

V. V. Sumbayev[1], *I. M. Yasinska*[1], *I. Goncalves Silva*[1], *S. S. Sakhnevych*[1], *E. Fasler-Kan*[2], *B. F. Gibbs*[1], *R. Hussain*[3], *G. Siligardi*[3], *W. Fiedler*[4];
[1]*University of Kent and Greenwich, Chatham Maritime, United Kingdom,* [2]*University Hospital Bern, Inselspital, Bern, Switzerland,* [3]*Diamond Light Source, Didcot, United Kingdom,* [4]*University Medical Centre Hamburg-Eppendorf, Hamburg, Germany.*

Acute myeloid leukemia (AML) is a blood/bone marrow cancer which is a severe and very often a fatal systemic malignancy. Cancerous blood cells are capable of escaping host immune surveillance by inactivating cytotoxic lymphoid cells (natural killer (NK) cells and cytotoxic T lymphocytes (CTLs)). Our work is focused on understanding molecular mechanisms underlying this pathophysiological process. We discovered a fundamental molecular pathway which includes ligand-induced activation of plasma membrane associated receptor latrophilin 1 leading to increased translation and exocytosis of the immune receptor Tim-3 (T cell immunoglobulin and mucin domain containing protein 3) and its ligand galectin-9. This occurs via protein kinase C and mammalian target of rapamycin (mTOR) pathways. Tim-3 is directly involved in galectin-9 secretion and can also be released in a free soluble form. Galectin-9 impairs the anti-cancer activity of NK cells and kills CTLs. Soluble Tim-3 prevents secretion of interleukin-2 (IL-2) required for NK cell/CTL activation as cytotoxic cells. These results were validated in *ex vivo* experiments using primary samples from AML patients. Importantly, activity of this pathway was detected in other types of cancer including breast and colorectal cancer. A fundamental Tim-3/galectin-9 secretory pathway is one of the crucial biochemical mechanisms determining the capability of human AML cells to escape host immune surveillance.

P.B1.03.19

ABCG1 modulates macrophage polarization in human monocyte-derived macrophages

D. Unuvar Purcu[1], *S. Gunalp*[2], *M. Altunay*[2], *G. Baslar*[3], *D. Sag*[3,4];
[1]*Department of Molecular Medicine, Health Sciences Institute, Dokuz Eylul University, Izmir, Turkey,* [2]*Izmir International Biomedicine and Genome Institute, Dokuz Eylul University, Izmir, Turkey,* [3]*Izmir Biomedicine and Genome Center, Izmir, Turkey,* [4]*Department of Medical Biology, School of Medicine, Dokuz Eylul University, Izmir, Turkey.*

The ATP-binding cassette transporter G1 (ABCG1) mediates efflux of cellular cholesterol to high-density lipoprotein (HDL) particles. Recently, we have shown that the absence of ABCG1 inhibits tumour growth in mice by modulating macrophage function within the tumour. In the absence of ABCG1, macrophages shift from a tumour-promoting M2 phenotype to a tumour-fighting M1 phenotype within the tumour and kill tumour cells directly. Conforming a similar tumour fighting potential of ABCG1 in human macrophages is the next crucial step to determine if ABCG1 can be a molecular target in cancer immunotherapy. To this end, we inhibited ABCG1 expression in human peripheral blood monocyte-derived macrophages by RNA interference before the macrophages were polarized to M1 or M2 phenotypes by the stimulation with LPS/IFNγ or IL-4, respectively. The expression of M1 and M2 markers were analysed by qPCR. The inhibition of ABCG1 by siRNA significantly increased the expression of the M1 markers IDO1, CXCL10, CD64 and TNF after LPS/IFNγ stimulation. In contrast, the inhibition of ABCG1 by siRNA significantly reduced the expression of the M2 markers MRC1, TGM2 and CD163 after IL-4 stimulation. Our results indicate that ABCG1-deficiency promotes macrophage polarization to an M1 phenotype in human monocyte-derived macrophages. These findings suggest that ABCG1 in macrophages could be a potential new target to modulate macrophage polarization for cancer immunotherapy.
FP7 Marie Curie/TUBITAK Cofunded Brain Circulation Scheme Fellowship (115C074, to D.S)

P.B1.03.20

Identification of high affinity TCRs directed against lineage-specific antigens expressed by solid tumors

R. A. van Amerongen*, R. S. Hagedoorn, M. G. Kester, M. van der Meent, D. Remst, D. M. van der Steen, S. Tuit, P. A. van Veelen, F. Falkenburg, M. H. Heemskerk;
Leiden University Medical Center, Leiden, Netherlands.*

Targeting lineage-specific antigens by T-cell receptor (TCR)-based therapies is an interesting option for solid tumor types that often require a complete resection of the organ. Examples are late stage prostate and ovarian cancer. These lineage-specific antigens should be expressed in both tumor tissues and the healthy counterpart. Hypothetically a more homogeneous expression of lineage-specific antigens in the target population can be expected, compared with tumor-associated targets that are mostly heterogeneously expressed. We have selected a group of lineage-specific antigens per tumor type, using publically available RNAseq databases of both healthy (HPA/GTEx) and tumor samples (TCGA). Examples of prostate lineage-specific genes included in this study are kallikrein-2, -3 and -4. To identify potential T-cell epitopes from these lineage-specific genes, peptide elution experiments were performed on resected tumors, tumor cell lines or transduced cell lines. Peptides binding in different common HLA molecules were selected, synthesised and confirmed, and peptide-MHC (pMHC) multimers were produced. In PBMCs of HLA-typed individuals these pMHC multimers were used to enrich for peptide-specific $CD8^+$ T-cells. We aim to identify potent high affinity T-cells by searching within the allo-HLA repertoire. Initially, over 3000 pMHC multimer positive $CD8^+$ T-cells were collected via single-cell sorting. T-cell clones that specifically recognized peptide loaded target cells, as well as target cells expressing endogenously processed antigen, were selected for further screenings. Using cell panels consisting of different healthy and malignant cell types, safety and efficacy screenings were conducted. Altogether, promising lineage-specific T-cell clones were identified, recognizing both tumor cell lines and primary tumor material.

P.B1.03.21

Adoptive T cell transfer combined with DC vaccination in patients with metastatic melanoma

S. L. Wickström[1], *T. Lövgren*[1], *M. Wolodarski*[1], *U. Edbäck*[1], *E. Martell*[2], *K. Markland*[1], *M. Nyström*[1], *A. Lundqvist*[1], *H. Jacobsson*[2], *J. Hansson*[1], *G. Masucci*[1], *R. Tell*[1], *I. Poschke*[3], *J. Mattsson*[1], *R. Kiessling*[1];
[1]*Karolinska Institutet, Stockholm, Sweden,* [2]*Karolinska Univeritets sjukhuset, Stockholm, Sweden,* [3]*German Cancer Research Center, Heidelberg, Germany.*

Adoptive T-cell therapy (ACT) has been reported to induce clinical responses in up to 70% of stage IV melanoma patients. The aim of the MAT02 trial is to investigate the effect of adoptive transfer of autologous, tumor infiltrating lymphocytes (TIL) with or without autologous dendritic cell (DC) vaccination in patients with advanced melanoma. The anti-tumor response will be evaluated and the transferred T cells will be characterized in regard to phenotype and function. This study is a single center, open-label, two-armed, phase I trial. Five patients are assigned to each cohort (A or B) and receive ACT of autologous TIL without (A) or with (B) autologous tumor loaded DC i.c. Radiological evaluation is performed four weeks after the last DC administration. Cohort A: patients received only TIL has been completed and the treatment had limited expected toxicity. All treated patients showed a mixed response or stable disease. However, these responses were not durable. Cohort B: received the combined treatment of TIL and DC-vaccine, three patients have completed the treatment, 29-33 billion TIL and were administered five DC-vaccinations. All 3 patients responded; one with a complete response which is still ongoing (> 15 mo). Two patients showed a partial response, one with a long lasting response (> 24 mo) and one with a short response. We expect to include another two patients before the summer of 2018. Adoptive T cell therapy combined with DC-vaccination is safe and can result in complete clinical response in patients who have failed on checkpoint therapy.

P.B1.04 Tumor vaccination principles and Immunotherapy - Part 4

P.B1.04.01

Media evaluation for production and expansion of anti-CD19 chimeric antigen receptor (CAR) T cells

R. Alnabhan[1,2]*, J. Mattsson*[3,4]*, M. Uhlin*[1,4]*, I. Magalhaes*[3]*;*
[1]Division of Surgery, Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden, [2]King Abdullah International Medical Research Center, Riyadh, Saudi Arabia, [3]Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden, [4]Department of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden.

The use of CD19 chimeric antigen receptor (CAR) T cells to treat B cell malignancies has been showing beneficial clinical outcomes. The production of CD19 CAR T cells usually involves serum supplementation. Currently, multiple manufacturers provide serum-free media that can be used without serum supplementation. Therefore, it becomes important to evaluate the production of CD19 CAR T cells with and without serum supplementation. Methods: T cells from buffy coats were cultured in AIM-V and TexMACS supplemented with 5% human serum (A5% and TM5%), and in TM without serum. Cells were activated with OKT3 and expanded in IL-2. Viral transduction was performed by spinoculation in RetroNectin-coated plates. We evaluated CD19 CAR T cell viability, expansion, transduction rate, phenotype and cytotoxicity. Results: CD19 CAR T cells expanded in A5% and TM5% showed significantly better viability and higher fold expansion than cells expanded in TM. TM promoted the expansion of $CD8^+$T cells and effector phenotype of CD19 CAR T cells. The transduction rate and the cytotoxicity were comparable between the different media. TM and TM5% promoted higher frequency of $CD107a^+$cells, while A5% promoted higher frequency of $IL\text{-}2^+$and $IL\text{-}17^+$ cells. CD19 CAR exhibited co-expression of exhaustion markers including; $TIM\text{-}3^+LAG\text{-}3^+$ and/or $TIM\text{-}3^+PD\text{-}1^+$. Conclusion: Our results indicate that the addition of serum promotes improved CD19 CAR T cells expansion and viability *in vitro*. Different culture media promoted different phenotypes of CD19 CAR T cells, which warrants further assessment in clinical settings. Overall, culture medium is a key factor that impacts CD19 CAR T cell.

P.B1.04.02

Development and preclinical testing of simultaneous dual engagement CARs (SiDECARs) against Acute Myeloid Leukemia

I. E. Balabanov*, H. M. Van Santen;*
Center for Molecular Biology "Severo Ochoa", Madrid, Spain.

Introduction: The use of immunotherapy in the treatment of cancer has provided clear benefits for patients. Still, exploitation of its full potential is hampered by, amongst other reasons, the severe side effects that can be caused by therapy-induced immune responses against healthy tissues of the patient. In the case of adoptive cell therapy with T cells, expressing chimeric antigen receptors (CARs) that recognize tumor-associated antigens, these off-tumor on-target effects are mainly due to the paucity of truly tumor-specific antigens. One way to improve tumor recognition capabilities of T cells is to generate CARs that require binding of two different ligands to initiate T cell activation.
Materials & Methods: We have generated bispecific CARs targeting two AML-associated antigens by appending ScFv-chains, recognizing these antigens, to the TCR. These receptors were designed with different intracellular signaling chains with the aim to find a balance between ligand binding affinity and intracellular signaling capacity, in order to promote optimal dual recognition-dependent T cell activation.
Results: cDNAs encoding the AML-specific antibodies were cloned and ScFv were successfully generated. The proposed CAR constructs were created through seamless cloning techniques and expressed in mouse and human primary T cells and T cell lines. Their capacities to induce T cell activation were investigated and compared using different human AML cell lines as targets.
Conclusions: This approach could be applied to other types of cancer.
This project is being funded by the Horizon 2020 program MSCA-ITN-2016 of the European Union with grant agreement GA721358 (ENACTI^{2}NG).

P.B1.04.03

Increased antigen loading in synthetic peptide vaccines - is more better?

T. R. Cooney[1]*, G. F. Painter*[1]*, I. F. Hermans*[2]*, G. F. Walker*[3]*;*
[1]Ferrier Research Institute, Victoria University of Wellington, Wellington, New Zealand, [2]Malaghan Institute of Medical Research, Wellington, New Zealand, [3]University of Otago, Dunedin, New Zealand.

Vaccines that generate T cell responses can be used to treat non-communicable diseases such as cancer. Peptide vaccines have considerable advantages compared to protein or whole cell based alternatives as they are highly defined, possess a good safety profile and are more amenable to optimisation. A major limitation of peptide vaccines is their lack of immunogenicity, however, this can be overcome through the incorporation of immune stimulants (i.e. vaccine adjuvants). Vaccine immunogenicity can be further increased via the chemical conjugation of a peptide to an adjuvant such as those which activate invariant natural killer T (iNKT) cells. Subunit vaccine designs which are limited to a 1:1 molar ratio of antigen:adjuvant ratio are not considered to be optimal with most vaccine formulations containing a higher ratio of peptide antigen compared to the adjuvant. To investigate the importance of high antigen loading we have employed a dendrimer scaffold which can be synthesised at a multi-generational level, to provide either 2, 4, 8, 16 or more antigen attachment sites branching from a singular adjuvant. Utilisation of a multi-generational branched dendrimer scaffold will enable investigation towards the hypothesis that higher antigen loading will produce more efficacious vaccines.

P.B1.04.04

Conventional $CD4^+$ T cells present bacterial antigens to induce cytotoxic and memory $CD8^+$ T cell responses.

A. Cruz-Adalia*, G. Ramirez Santiago, J. Osuna, M. Torres Torresano, E. Veiga;*
CNB/CSIC, Madrid, Spain.

Bacterial phagocytosis and antigen cross-presentation to activate $CD8^+$ T cells are principal functions of professional antigen presenting cells. However, conventional $CD4^+$ T cells also capture and kill bacteria from infected dendritic cells in a process termed transphagocytosis (also known as transinfection). Here, we show that transphagocytic T cells present bacterial antigens to naive $CD8^+$ T cells, which proliferate and become cytotoxic in response. $CD4^+$ T-cell-mediated antigen presentation also occurs in vivo in the course of infection, and induces the generation of central memory $CD8^+$ T cells with low PD-1 expression. Moreover, transphagocytic $CD4^+$ T cells induce protective anti-tumour immune responses by priming $CD8^+$ T cells, highlighting the potential of $CD4^+$ T cells as a tool for cancer immunotherapy.

P.B1.04.05

Intracellular localization of the negative checkpoint regulator VISTA in Antigen Presenting Cells

L. Dübbel[1]*, K. W. Koch*[1]*, N. H. Meyer*[2]*;*
[1]Biochemistry, Department of Neuroscience, University of Oldenburg, Oldenburg, Germany, [2]Dermatology and Allergology, Department of Human Medicine, University of Oldenburg, Oldenburg, Germany.

In the recent past, blocking of the negative checkpoint regulators *Programmed Death-1* (PD-1) and *Cytotoxic T-lymphocyte Antigen-4* (CTLA-4) showed groundbreaking results in the immunotherapy of cancer.
The negative checkpoint regulator *V-domain immunoglobulin (Ig)-containing suppressor of T-cell activation* (VISTA) is a new potential target for cancer immunotherapy. In mice, the anti-VISTA antibody treatment is not only increasing tumor specific T-cells in the periphery, but also the infiltration, proliferation and effector function of tumor reactive T-cells in the tumor microenvironment. Consequently, an anti-VISTA antibody was recently approved for a phase I clinical trial.
The surface expression pattern of VISTA is clearly distinct from other negative checkpoint regulators. Intriguingly, VISTA is expressed on both, T-cells and Antigen Presenting Cells, with very high expression on tumor-infiltrating immune cells. Since model negative checkpoint regulators are exclusively expressed on T-cells the function of VISTA on Antigen Presenting Cells remains unknown.
We thus investigated the expression of VISTA on murine *bone marrow derived macrophages* (BMDM) and human monocyte-derived macrophages. Particularly, we analyzed the localization of VISTA in different types of vesicles using immunofluorescence microscopy. In addition, we isolated different cell fractions and organelles to analyze the subcellular localization of VISTA in Antigen Presenting Cells.
Our results show that VISTA co-localizes with several vesicle markers (SNAP-25, CD71, Perforin) and is released to the cell surface upon stimulation. A similar mechanism has been previously suggested for CTLA-4.
Overall, we conclude that VISTA storage in vesicles allows a fast response of APCs to immunogenic stimuli.

P.B1.04.06

Nanobodies as tools for targeting of Adeno-associated viruses to target-expressing cells

A. Eichhoff[1], *T. Eden*[1], *F. Haag*[1], *S. Adriouch*[2], *F. Koch-Nolte*[1];
[1]*Institute of Immunology, UKE, Hamburg, Germany,* [2]*Institute of Medicine and Pharmacy, Inserm U1234, Rouen, France.*

Adeno-associated viruses (AAVs) are widely used as vectors in gene and tumor therapy to treat various diseases. A limiting factor for successfull gene delivery without side effects is the broad tropism of AAV serotypes, i.e. the parallel infection of several tissues and cell types.
Here, we show that Nanobodies - the single binding domain of camelid heavy chain antibodies - can be used as ligands to target AAVs to specific cells. Nanobodies provide high specificity and stability, and their small size allows easy reformatting as fusion proteins.
The ectoenzymes CD38 and CD296 and the P2X7 ion channel were evaluated as target receptors for AAVs. In one strategy, the membrane protein-specific nanobody was genetically inserted into an exposed surface loop of the viral capsid protein VP1 of AAV2^{RA} (a variant containing two mutations of arginines to alanines that inhibit binding to HSPG). The presentation of the nanobody on the viral capsid resulted in specific transduction of cells expressing the target with GFP-encoding AAVs.
As a second strategy, the membrane protein-specific nanobody was genetically fused via a flexible peptide linker to an AAV1/2 dual-reactive nanobody, thereby generating bispecific nanobody-based adaptor proteins. These adaptors strongly and specifically enhanced the transduction of cells expressing CD38, CD296, or P2X7 by both AAV1 and AAV2^{RA}.
These results provide proof of principle for nanobody-based strategies to enhance the cell specificity of AAVs and provide a basis for new approaches to optimize Adeno-associated viral vectors for gene and tumor therapy.

P.B1.04.07

Regulation of the NK cells melanoma cytotoxic cross talk by nanotechnology assisted p53 reactivation

c. garofalo[1], *M. Naddeo*[2], *V. Ventura*[1,3], *L. Izzo*[4], *G. Selivanova*[5], *D. Pappalardo*[2], *E. Carbone*[1,5];
[1]*Tumor Immunology and Immunopathology Laboratory, Department of Experimental and Clinical Medicine, University Magna Graecia of Catanzaro, Catanzaro, Italy., catanzaro, Italy,* [2]*Department of Science and Technology, University of Sannio, via dei Mulini 59/A, 82100 Benevento, Italy., Benevento, Italy., Italy,* [3]*Department of Health Sciences, University "Magna Græcia" of Catanzaro, Catanzaro, Italy, Catanzaro, Italy,* [4]*Department of Biotechnology and Life Science, University of Insubria, via J. H. Dunant, 3, 21100 Varese - Italy, Varese Italy, Italy,* [5]*Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, 17177, Stockholm, Sweden., Stockholm, Sweden.*

Introduction: PRIMA-MET and RITA are small molecules able to restore p53 function and induce tumor cells apoptosis. We and others demonstrated that these molecules enhance the NK cell-mediated recognition of solid tumors by promoting the expression of NKG2D ligands on tumor cell surface. To improve the pharmacological effect of these compounds on tumor cells, and increase their solubility and transport efficiency in the body fluids,we analyzed a panel of biocompatible polymers for their capability to form nanoparticles able to deliver a drug cargo inside tumor cell lines. We found that novel designed FITC-conjugated $mPEG_{2K}$-$(PD,LLA)_2$ was able to efficiently deliver paclitaxel anti-cancer drug inducing tumor cell death and we decide to use it as carrier for p53 reactivating small molecules (PRIMA-1MET and RITA)
Materials and Methods: Primary melanoma cells were treated with FITC-labeled PRIMA-1MET-loaded nanoparticles and analysed for the expression of NK activating/inhibitory molecules by FACS.
Results: Our preliminary data showed that FITC-nanoparticles loaded with PRIMA-1MET were detected in the melanoma cytoplasm after 6 days of treatment. Moreover, the treatment increased MICA while it reduced the PD-L1 expression on melanoma cells.
A new formulation of FITC-nanoparticles loaded with RITA has been generated.
Conclusions: The new formulation of PRIMA-1MET goes into the melanoma cells and change their immune phenotype.

P.B1.04.08

Virus specific T lymphocyte expansion for adoptive immunotherapy

M. Grau-Vorster[1,2,3], *M. López-Montañés*[1,2], *A. del Mazo-Barbara*[1,2], *S. Querol*[1,2], *J. Vives*[1,2,3], *I. Oliver-Vila*[1,2], *F. Rudilla*[1,2];
[1]*Blood and Tissue Bank, Barcelona, Spain,* [2]*Vall d'Hebron Research Institute, Barcelona, Spain,* [3]*Autonomous University of Barcelona, Barcelona, Spain.*

Adoptive immunotherapy with virus-specific T lymphocytes (VST) can prevent immunodeficient patients from virus reactivation or *de novo* infection. However, this treatment has several limitations, such as HLA-compatibility restriction, high costs and time required in the production of personalised medicines. These limitations can be overcome by the generation of a third-party VST bank through large-scale expansion compliant with Good Manufacturing Practice (GMP) standards. Cytomegalovirus (CMV) peptides were used to stimulate lymphocytes specifically against these antigens. Readouts after expansion included cell counting, purity, in terms of $CD8^+$ and $CD4^+$, and specificity (interferon gamma (IFNγ) expression). Moreover, product characterisation through immunophenotype, ELISPOT, degranulation, cytotoxicity and alloreactivity assays were performed. Physiological VST stimulus was tested, consisting of CMV pp65 peptide-pulsed autologous dendritic cells (DCs) derived from monocytes. Furthermore, anti-CD3+anti-CD28 antibodies were added in order to enhance VST proliferation. The combination of cytokines IL-2+IL-7+IL-15 in a G-Rex cell culture system resulted in optimum conditions for VST expansion. The use of DCs for VST activation and the addition of anti-CD3+anti-CD28 in the presence of IL-2 resulted in greater than 40-fold expansion at day 14 and >17% of specificity was achieved. After a purification step based on Cytokine Capture System (Miltenyi Biotec) 90% purity of VST was obtained. A GMP scalable process and with high values of VST was successfully defined. The phenotypic analysis as well as the functionality and safety of the final product have been tested. A further improvement to the approach would consider specificity for other viruses.

P.B1.04.09

Anti-tumoral potential of a granulysin-containing protein product directed against the carcinoembryonic antigen*

R. Ibáñez[1], *P. Guerrero*[1], *S. Al-Wasaby*[1], *R. Navarro*[2], *D. de Miguel*[1], *O. Gonzalo*[1], *B. Conde*[1], *L. Martínez-Lostao*[3], *R. Hurtado-Guerrero*[4], *L. Sanz*[2], *A. Anel*[1];
[1]*University of Zaragoza, Zaragoza, Spain,* [2]*University Hospital "Puerta de Hierro", Majadahonda, Spain,* [3]*Clinical University Hospital "Lozano Blesa", Zaragoza, Spain,* [4]*Institute for Biocomputation and Physics of Complex Systems (BIFI), Zaragoza, Spain.*

Introduction: Granulysin is a protein present in the granules of human CTLs and NK cells, with cytolytic activity against microbes and tumors. Previous work from our group demonstrated the *in vivo* antitumoral activity of intratumoral injection of recombinant granulysin in two animal models of tumor development, breast adenocarcinoma and multiple myeloma. In the present work we have developed a granulysin-containing protein product directed against the carcinoembryonic antigen (CEA), commonly expressed in colon adenocarcinomas. Material and Methods: 1) Recombinant granulysin or the protein product were tested against cells negative for CEA expression (Jurkat), or on CEA^+ cells (HT-29, SW1222, MKN45 or HeLa-CEA) and cell death was analyzed by annexin-V staining and 7-AAD incorporation. 2) Granulysin and the protein product were tested as a treatment in *in vivo* models of tumor development, based in subcutaneous injection of $3x10^6$ colon carcinoma HT-29 cells or HeLa-CEA transfected cells in groups of immunodeficient athymic mice. In the HT-29 model, injections were performed intratumorally, and in the HeLa-CEA model, injections were performed systemically (intraperitoneal). Results: We demonstrated that the cytotoxicity of the protein product against several CEA^+ cell lines *in vitro* was higher than that of granulysin alone. After intratumoral injection, both granulysin and the protein product were able to inhibit HT-29 tumoral growth more than 60%, demonstrating the *in vivo* bioactivity of the protein product. Finally, the protein product demonstrated a higher *in vivo* therapeutic effect against tumors derived from HeLa-CEA cells than granulysin alone after systemic administration. *The "protein product" is under patent tramitation

P.B1.04.10

Humanized heavy chain antibodies based on CD38-specific nanobodies effectively inhibit tumor growth in a systemic human lymphoma xenograft model

J. Hambach, L. Schriewer, N. Schuster, B. Albrecht, K. Petry, D. Aytan, J. Röckendorf, P. Bannas, ***F. Koch-Nolte****;
University Medical Center Hamburg, Hamburg, Germany.*

The cell surface ecto-enzyme CD38 is a target for the treatment of hematological malignancies. Nanobodies derived from camelid heavy chain antibodies are highly soluble and can bind to epitopes that are not accessible for conventional antibodies. Other advantages of nanobodies include their better tissue penetration *in vivo*, and the facile construction of bi- or multi-specific biologicals by genetic fusion (1). We have generated humanized heavy chain antibodies by fusion of CD38-specific nanobodies to the hinge and Fc-domains of wild type and engineered human IgG1. Some of these heavy chain antibodies mediate potent complement dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) of CD38-expressing tumor cell lines in vitro. In vivo some of the heavy chain antibodies reduced the growth of systemic CA46 lymphomas in tumor-bearing SCID mice more effectively than daratumuab, the benchmark conventional human IgG1 in the clinic.(1) Bannas P, Hambach J, Koch-Nolte F. 2017. Nanobodies and Nanobody-Based Human Heavy Chain Antibodies As Antitumor Therapeutics. Front Immunol. 8:1603.

P.B1.04.11

Enforced expression of a constitutively active form of GSK3β as a novel treatment to combat melanoma through facilitation of dendritic cell differentiation and activation

M. Lopez Gonzalez[1], *R. van de Ven*[1], *D. Oosterhoff*[1], *J. Jan Lindenberg*[1], *H. de Haan*[1], *W. Dong*[2], *V. W. van Beusechem*[1], *T. D. de Gruijl*[1];
[1]*Cancer Center Amsterdam, Amsterdam, Netherlands,* [2]*ORCA Therapeutics BV, Amsterdam, Netherlands.*

Even though immune checkpoint blockade has increased the overall survival of melanoma patients considerably, not all patients respond to this treatment, in part due to a lack of T-cell infiltration in the tumor fields. Activation of the *Wnt signaling* pathway has been identified as a molecular mechanism underlying this lack of immune infiltration, resulting in a lack of cross-presenting dendritic cells (DCs), which in turn are vital for the chemo-attraction and activation of tumor-infiltrating T-cells. β-catenin levels can be directly regulated by glycogen synthase-3-beta (GSK3β), an enzyme involved in various signaling pathways. When GSK3β is activated it will phosphorylate β-catenin which will lead to its inactivation and degradation.
Here, we report that modulation of GKS3β at the level of DCs plays a key role in their differentiation and maturation, and that overexpression of the constitutively active form of GKS3β (CA.GSK3β) renders DCs refractory to the suppressive effect of IL-10, an important suppressive factor released by metastatic melanoma, and of melanoma-derived supernatants. Moreover, the inhibition of GKS3β in melanoma cells increases their suppressive effect on monocyte-to-dendritic cell differentiation. Conversely, the enforced over-expression of CA.GSK3β reduces this suppressive effect and drives DC development to a type-1 T-cell-activating phenotype.
Based on our findings, we hypothesize that the enforced expression of CA.GSK3β at the melanoma tumor site may enhance the infiltration and activation of DC and T-cells and thereby render previously resistant tumors sensitive to immune checkpoint blockade.
This work is supported by the European Union Horizon2020 ITN-EID grant 643130 "VIRION".

P.B1.04.12

Use of alpha technology to identify novel small molecules that specifically inhibit LAG-3 binding to its ligand HLA-DR

G. H. Mason[1], *B. J. MacLachlan*[1], *A. Greenshields Watson*[1], *F. Triebel*[2], *D. K. Cole*[1,3], *A. Godkin*[1];
[1]*Division of Infection & Immunity, Cardiff, United Kingdom,* [2]*Immutep, Orsay, France,* [3]*Immunocore Ltd, Abingdon, United Kingdom.*

Introduction: $CD4^+$ helper T-cells express a series of activating and inhibiting co-receptors. These play a role in both autoimmunity and cancer immunity. Immune checkpoint inhibitors (ICI) which target molecules mediating inhibition (e.g. PD1, CTLA-4) may result in enhanced anti-tumour immunity. Lymphocyte activation gene-3 (LAG-3) is a negative regulator of T-cells that has not so far been successfully targeted by monoclonal antibodies. LAG-3^+ T-cells within tumours have been shown to be immunosuppressive and are associated with poor prognosis. As an alternative to expensive antibody based therapies, we conducted a small molecule library screen to identity lead compounds which may interfere with the interaction of LAG-3 with its ligand HLA class II.
Materials and Methods: Alpha technology, a bead based assay for the detection of protein:protein interactions (PPI), was used to screen a 50,000 compound library against i) LAG-3-HLA II ii) irrelevant control PPI using TCR:pHLA.
Results: 50 compounds which specifically block the interaction between LAG-3 and HLA II have been identified as true assay hits which block the LAG-3-HLA II interaction without blocking an irrelevant PPI.
Conclusions: Having identified hit compounds, these compounds are being tested using a LAG-3 expressing reporter cell line. Generation of a novel LAG-3-HLA II inhibitor, will enable us to learn more about the role of LAG-3 in disease setting, and aid the development of novel therapeutics.

P.B1.04.13

B cell lineage-specific transcription coactivator BOB1 is indispensable for multiple myeloma cell survival and allows for superior TCR-based targeted therapy

R. M. Reijmers, *A. D. Meringa, L. Jahn, F. Falkenburg, M. H. Heemskerk;*
Leiden University Medical Center, Leiden, Netherlands.

Although still incurable, much progress has been made in the treatment of multiple myeloma (MM). Recent advances in immunotherapy have contributed substantially, of which the most promising, T cells modified to express a chimeric antigen receptor (CAR) directed against B cell maturation antigen (BCMA). Another approach is introducing a transgenic T cell receptor (TCR) into cytotoxic T cells. Recently, for MM, we successfully demonstrated *in vivo* efficacy of a transgenic TCR targeting the transcription coactivator octamer binding protein-1 (BOB1) in the context of HLA-B*07.02. BOB1 is a B cell lineage specific protein that is highly expressed in all B cell malignancies, including MM. Like rituximab (anti-CD20) treatment, targeting BOB1 will only affect the B cell lineage, which makes it attractive for immunotherapy with high *on*-target and low *off*-tumor effects. This prompted us to further explore the significance of BOB1 in MM. To this end, we applied CRISPR/Cas9 to disrupt BOB1 expression in several MM cell lines. Remarkably, upon single-cell sorting and DNA sequencing, all targeted clones revealed in-frame deletions only. We are currently extending these findings to other B cell malignancies and study the functionality of the in-frame mutated BOB1 variants on target gene expression, and study the effect on *in vitro* and *in vivo* growth. Together, these data suggest that BOB1 is indispensable for myeloma cell survival, which identifies BOB1 as a superior target for TCR-based immunotherapy. This work was financially supported by Bellicum Pharmaceuticals.

P.B1.04.14

Identifying immunological properties of natural products for the treatment of chemoresistant tumors and bacterial pathogens

L. Richter[1], *M. Frank*[2], *P. Proksch*[2], *S. Scheu*[1];
[1]*Heinrich Heine University, Institute of Medical Microbiology and Hospital Hygiene, Duesseldorf, Germany,* [2]*Heinrich Heine University, Institute of Pharmaceutical Biology and Biotechnology, Duesseldorf, Germany.*

Introduction: The most abundant human diseases worldwide include cancer and bacterial infections. To overcome the development of resistances against cytostatics and antibiotics, there is a rising need to find new drugs. Natural products comprise many untested highly bioactive molecules inspiring medical research. We are looking for compounds that modulate immune effector functions and additionally target tumors and pathogens, thus reducing the risk of resistances. Material and methods: A library with 240 natural products derived from endophytic fungi and marine sponges undergoes different screenings to determine promising compounds. After having completed cytotoxicity screenings, we are currently using an IL-12p40 fluorescence reporter mouse line to test selected natural products for their ability to induce or enhance expression of IL-12 in macrophages or dendritic cells via flow cytometry and ELISA. The thus identified immune activating compounds will be further interrogated in T cell activation assays for their potential to promote T cell priming by dendritic cells. Results: We have identified 41 natural products that are non-toxic to immune cells but simultaneously toxic to tumor cells or pathogens. For application of the aforementioned IL-12p40 assay, cell culture and FACS staining conditions have been successfully established and multifunctional natural products will be screened for immune activation. Conclusions: Promising multifunctional, immune activating natural products will further be biochemically optimized for immune modulatory effectivity and tested in *in vivo* tumor and infection mouse models. This work is funded by the German Research Foundation (DFG).

P.B1.04.15

Human γδT cells: Presentation Of Tumour Antigens And Induction Of Anti-Tumour Immunity

T. Rus[1], *M. Eberl*[1,2], *B. Moser*[1,2];
[1]*Cardiff university, School of medicine, Cardiff, United Kingdom,* [2]*Systems Immunity Research Institute, Cardiff, United Kingdom.*

An important discovery that activated human blood γδT cells behave as professional antigen-presenting cells (γδT-APCs) has highlighted their potential use as cellular vaccines. γδT-APCs are ideal candidate for immunotherapy as they are non-MHC-restricted and they do not mount unwanted/unpredicted cross-reactivities in patients. They are capable of inducing potent antigen-specific and MHC-restricted responses that may boost anti-tumour cytotoxic T cell responses. Lastly, they can be generated in large numbers ($>10^9$) *in vitro* from small blood samples and predominantly secrete proinflammatory cytokines (IFNγ, TNFα), which may help overcome immune inhibitory conditions frequently associated with tumours. It has been shown that γδT-APCs can excellently process microbial antigens (influenza, CMV, Mtb), including peptides, whole proteins and cell extracts, and induce strong responses in responder αβT cells. This study focuses on the ability of γδT-APCs to process and present tumour antigens, including NY-ESO-1 and 5T4, which are frequently expressed in malignant melanomas and breast cancer but not in healthy tissue.
This is accomplished through the production and purification of recombinant protein from *E.coli*, and antigen-specific CD8+ T cells from PBMC in an autologous system. These are then used to examine the functionality of γδT-APCs in *in vitro* APC assays. Demonstration that γδT-APCs are capable of inducing strong tumour-specific CD4+ and CD8+ αβT cell responses is a prerequisite for carrying our first-in-man clinical trials. Further work will include localisation and generation of γδT-APCs from cancer tissues, as well as the generation of a mouse immunotherapy model to test the benefits of γδT-APCs as a cellular vaccine.

P.B1.04.16

PD-1 blockade impairs the anti-tumor activity of innate immune cells stimulated with TLR9 agonist

C. Storti[1], *M. Sommariva*[1], *M. De Cesare*[2], *E. Tagliabue*[3], *A. Balsari*[1,3], *L. Sfondrini*[1];
[1]*Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Milan, Italy,* [2]*Molecular Pharmacology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy,* [3]*Molecular Targeting Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.*

Introduction: TLR agonists are known to activate innate immune cells and can exert anti-tumor activity. We previously reported that locoregional administration of CpG-ODN, synthetic TLR9 agonist, inhibited IGROV-1 human ovarian cancer cell growth in athymic nude mice, preclinical model devoid of T lymphocytes in which anti-tumor immune response is mediated only by innate immune system. PD-1 receptor is expressed by activated T lymphocytes and also by innate immune cells. Interaction of PD-1 with PD-L1 or -2 blunts immune response, but specific antibodies blocking PD-1 can re-activate it. Therefore, combinations of TLRs therapies with anti-PD-1 antibody may be a promising new therapeutic strategy.
Methods: Nude mice were intraperitoneally xenografted with IGROV-1 cells and locally treated with CpG-ODN in combination with anti-PD-1 antibody. Macrophages were depleted by liposomes-containing clodronate. Immunocompetent mice injected with B16 melanoma and 4T1 breast cancer cells were treated as above.
Results: CpG-ODN/anti-PD-1 antibody combination in IGROV-1-injected mice was found to be less efficacious than CpG-ODN alone, independently from the treatment schedules and anti-PD-1 clone used. The same combination in B16- or 4T1-injected immunocompetent mice did not reveal a similar effect. Administration of anti-PD-1 antibody immediately after IGROV-1 tumor injection determined tumor growth acceleration. Immunohistochemical analysis showed an increase of $Arg1^+$ intratumoral macrophages in anti-PD-1 treated group. Macrophage depletion restored CpG-ODN antitumor effect when combined with anti-PD-1 antibody.
Conclusion: Our results suggest that blocking PD-1 pathway reduced the therapeutic efficacy of CpG-ODN probably due to an effect probably mediated by macrophages.
Supported by AIRC

P.B1.04.17

Estimation of antitumor effect of chemoimmunotherapy with methotrexate nanoconjugates and dendritic cell-based vaccines in MC38 murine colon carcinoma model

A. Szczygieł, *N. Anger, K. Węgierek, J. Mierzejewska, J. Rossowska, T. Gościczyński, M. Świtalska, E. Pajtasz-Piasecka;*
Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland.

Nanoconjugates of methotrexate (MTX) and hydroxyethyl starch (HES) are a new type of therapeutic compounds formed from certified drugs widely used in medicine. Conjugation of MTX with HES prolongs the half-life of MTX and reduces its side effects. In order to enhance the therapeutic effect, we combined MTX-HES nanoconjugate with bone marrow-derived dendritic cells (BM-DCs), which are considered to be capable to activate the immune system in the host.
To determine the antitumor effect of chemoimmunotherapy, mice with subcutaneously growing MC38 tumor intravenously received MTX or MTX-HES and three days later, immunotherapy started (peritumoral injections of mature BM-DCs). To evaluate the changes in percentage of tumor infiltrating lymphocytes and myeloid cells, three days after chemotherapy or seven days after immunotherapy, tumors were collected for multiparametric flow cytometry analyses.
On the 3rd day after chemotherapy in MTX-HES-group the highest percentage of $CD4^+$ and $CD8^+$ T cells infiltrating tumor tissue was observed, moreover in both groups the percentage of Tregs was drastically decreased. Supplementing therapy with BM-DC-vaccines intensified this effect, additionally an increase in the percentage of NK cells was also noticed. Infiltration of leukocytes were enhanced only after chemoimmunotherapy, moreover application of BM-DC-vaccines resulted in reduction of percentage of myeloid cells with suppressor activity.
Concluding, therapy with nanoconjugates and BM-DCs-vaccines resulted in efficient response against growing tumor. These findings demonstrate that methotrexate-nanoconjugate immunomodulates the antitumor response and this effect was beneficial for generation of proper immune response by dendritic cells.
The study was funded by National Science Centre, Poland (project no.2015/19/N/NZ6/02908).

P.B1.04.18

Sodium stibogluconate in conjunction with CD47-SIRPαcheckpoint blockade enables rituximab-mediated killing of B lymphoma cells by neutrophils

D. J. van Rees[1], *M. van Houdt*[1], *A. Tool*[1], *P. Verkuijlen*[1], *K. Schornagel*[1], *K. Franke*[1], *T. W. Kuijpers*[1,2], *T. K. van den Berg*[1,3], *H. L. Matlung*[1];
[1]*Sanquin Research and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands,* [2]*Emma Children's Hospital, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands,* [3]*Department of Molecular Cell Biology and Immunology, VU medical center, Amsterdam, Netherlands.*

Rituximab (Rmab) is used as a first-line treatment for CD20-expressing B cell malignancies. It is believed to act by a combination of direct and immune-mediated effects, including complement- and immune cell- dependent mechanisms. However, neutrophils, the most abundant effector cells mediating antibody-dependent cellular cytotoxicity (ADCC) are incapable of killing Rmab-opsonized B lymphoma cells. Instead, Rmab triggers neutrophil trogocytosis of CD20-containing plasma membrane fragments of the target cells, which is believed to render the tumor cells resistant against further Rmab-dependent destruction. Interestingly, we found previously that neutrophils exert an entirely novel type of cytotoxicity against antibody-opsonized solid cancer cells, designated *trogoptosis*, which actually involves a trogocytic process. Here, we demonstrate that the lack of cytotoxicity of neutrophils towards Rmab-opsonized B lymphoblastoid cells is not due to an inherent defect of neutrophils to destroy such target cells, but rather to intrinsic properties of the CD20-Rmab complex. Furthermore, we present evidence that a combination of CD47-SIRPα checkpoint blockade and the tyrosine phosphatase inhibitor sodium stibogluconate (SSG) are able to overcome the neutrophil defect to effectively kill B lymphoma cells in the presence of Rmab. This provides opportunities for improving the clinical efficacy of Rmab treatment in cancer.

P.B1.04.19

The supernatant of immature dendritic cells mediates RIP1-dependent apoptosis

Z. Varga, *E. Jakab-Racz, A. Szabo, E. Rajnavolgyi, G. Koncz;*
Department of Immunology, University of Debrecen, Debrecen, Hungary.

Dendritic cells (DCs) are known to engulf dead cells continuously and present antigenic fragments derived from infected cells and tumor antigens thus having the capacity of triggering naïve $CD8^+$ T cells. The cross-priming process has been known as a unique route to initiate classical T cell responses. *In vitro* generated moDCs have also been demonstrated to induce apoptosis in target cells.
We provide evidence here that supernatants of activated immature moDCs activated by PRR agonist (LPS, poly (I:C) or Cl-075) induces cell death on Jurkat cells. In contrast, the supernatant of mature DCs were less cytotoxic, which may effect on the adoptive DC therapies. TNF:Fc fusion protein inhibited the cytotoxic activity of DCs in contrary to Fas and TRAIL antagonists, suggesting it is a TNF dependent, but Fas and TRAIL independent process. In contrary to cytotoxic T cell-mediated killing, secreted vesicles from culture supernatants of moDC were not able to generate cell death. To define the molecular mechanisms of DC-mediated signaling we were able to detect RIP1-dependent cell death. Pretreatment of target cells with a pancaspase inhibitor (zVad) completely blocked moDC triggered apoptosis, but necroptosis inhibitor (nec-1) did not prevent this cell death.
In summary, our results indicate that the supernatant of immature dendritic cells induces RIP1-dependent apoptosis, which may be relevant for tumor immunotherapy broaden the plethora of cytotoxic mechanisms acting against tumoric cells.
The work is supported by NKFIH-K-125224 and by the GINOP-2.3.2-15-2016-00050 project. The project is co-financed by the European Union and the European Regional Development Fund.

P.B1.05 Tumor vaccination principles and Immunotherapy - Part 5

P.B1.05.01

From mono- to bivalent: increasing affinity of EGFR-specific target modules results in enhanced anti-tumor properties of the UniCAR system

S. Albert[1], *C. Arndt*[2], *S. Koristka*[2], *N. Berndt*[2], *R. Bergmann*[2], *A. Feldmann*[2], *M. Bachmann*[1,2,3];
[1]*University Cancer Center Dresden, Dresden, Germany,* [2]*Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Institute of Radiopharmaceutical Cancer Research, Dresden, Germany,* [3]*German Cancer Consortium (DKTK), partner site Dresden and German Cancer Research Center (DKFZ), Heidelberg, Germany.*

Introduction: Despite improvement of conventional cancer therapies, treatment of epithelial tumors needs to be further optimized. Especially chimeric antigen receptor (CAR)-modified T cells possess a tremendous immunotherapeutic potential. However, due to the risk of serious side effects and the lack of efficient control mechanisms, we established the switchable modular UniCAR system. This system is composed of two distinct components, T cells expressing a universal CAR (UniCAR) and exchangeable tumor-specific target modules (TMs). Based on the modular structure, engrafted T cells are inert in the absence of TMs and only switched on in their presence.
Materials and Methods: For redirection of UniCAR T cells to epithelial tumors, we recently generated a monovalent nanobody-based α-EGFR TM. After expression in CHO cells, the construct mediated an antigen-specific tumor cell lysis. To analyze whether the functionality can be improved by increasing the affinity, a bivalent α-EGFR-EGFR TM was established and compared to the monovalent counterpart.
Results: Due to raising the number of binding sites, the bivalent TM shows an increased avidity, higher levels of released pro-inflammatory cytokines and an improved killing capability *in vitro* and *in vivo*. Nevertheless, cells with an EGFR density comparable to physiological numbers of EGFR are not eliminated and, therefore, destruction of healthy tissues is rather unlikely.
Conclusions: Summing up, increasing the avidity of the TM enhances its functionality.

P.B1.05.02

Testing monoclonal antibodies blocking TGF-β1 production from human GARP/TGF-β1 complexes in mutant mice expressing a humanized form of Garp

***C. Bertrand**;*
de Duve Institute, Brussels, Belgium.

Regulatory T cells (Tregs) are important contributors to immunosuppression in tumors. Based on our previous work, we believe that TGF-β1 is a major player in Treg-mediated immunosuppression. We showed that Tregs activate TGF-β1 through a mechanism that implies transmembrane protein GARP and can be inhibited with blocking anti-GARP antibodies, which could thus serve as drugs to increase anti-tumor immune responses.
Our blocking antibodies bind h(uman) GARP, but not m(urine) GARP. We thus generated mice expressing a humanized version of Garp (*mGarp*$_{Hu137\text{-}139}$ mice) that can be bound by the blocking anti-hGARP antibodies.
In this project, our main objective is to assess the activity and toxicity of blocking anti-hGARP antibodies in preclinical models of cancer in *mGarp*$_{Hu137\text{-}139}$ mice.
Generation of *mGarp*$_{Hu137\text{-}139}$ mice was performed by subcontractors in two genetic backgrounds, namely C57BL/6 and DBA/2. Homozygous mutant mice were born to Mendelian ratios from heterozygous couples, and showed no gross anomaly after birth.
We show that the *mGarp*$_{Hu137\text{-}139}$ mutation does not affect the expression pattern of the *Garp* gene in the genetically manipulated mice, by comparison to wild-type littermates. Flow cytometry and qPCR analysis of various organs (thymus, blood, spleens, bone marrows, lymph nodes) indicate normal hematopoietic cell development and numbers. No signs of auto-immunity or general T cell activation is observed in mutant mice.
We verified that Tregs from mutant *mGarp*$_{Hu137\text{-}139}$ mice were able to produce active TGF-β1 upon *in vitro* stimulation, and that this production could be blocked with the blocking anti-hGARP antibodies.

P.B1.05.03

Towards a clinical grade CD137-based isolation and expansion of neoantigen-specific T-cells for tailored cancer adoptive cell therapies

***V. Bianchi**, S. Bobisse, R. Genolet, L. Kandalaft, G. Coukos, A. Harari;*
Ludwig Institute for Cancer Research, Lausanne, Switzerland.

Mounting evidence suggests that neoantigens tumor neo-antigens derived from non-synonymous somatic mutations represent ideal candidates to target with T-cell mediated immunotherapies. The selective isolation of low frequency neoantigen-specific T-cells in patients' TIL (Tumor Infiltrating Lymphocyte) and PBMC (Peripheral Blood Mononuclear Cell) bulk populations represents one of the major challenges in the development of personally tailored cancer T-cell therapies. A surface marker uniformly upregulated upon activation would facilitate the isolation of neoantigen-specific T-cells when cognate peptide-HLA multimers are not. The TNFR family member CD137 has been well characterized as a specific marker of TCR-induced activation of conventional CD8+ (and CD4+) T-cells. Following antigen-specific stimulation, CD137 expression on CD8+ T-cells peaks at 20-24h, therefore allowing the detection of viable T-cells displaying reactivity to the epitope of interest. Within the framework of our translational neoantigen discovery platform, we are in the process of developing a clinical-grade strategy for the *ex vivo* isolation and expansion of viable neoantigen-specific T-cells based on CD137 expression and flow-cytometric separation from patients' TIL and/or blood samples. The protocol is currently being validated on healthy donors PBMC samples with known viral and tumor T-cell reactivities and, in close collaboration with the Process Development team at CTE (Lausanne), will be performed with clinical grade compliant materials and reagents. By enriching for neoantigen-specific T-cells prior to infusion, it may be possible to improve the overall response rate achieved so far by unselected adoptive T-cell therapies.

P.B1.05.04

Preclinical Safety, Pharmacokinetics and Pharmacodynamics of BION-1301, a first-in-class antibody Targeting APRIL for the Treatment of Multiple Myeloma

***J. Dulos**;*
Aduro Biotech Europe, Oss, Netherlands.

BION-1301 is a first-in-class humanized antibody targeting APRIL (TNFSF13). A single-dose non-human primate (NHP) study administering intravenous BION-1301at 0.3, 3 and 30 mg/kg dose levels yielded PK parameters typical for IgG4 class antibodies and an absence of tolerability issues. PD analysis showed a statistically significant reduction in total IgA and IgM in a dose-dependent fashion. Consistent with previous observations in hAPRIL transgenic mice, BION-1301 reduced TNP-specific IgA and IgM in NHP in a dose-dependent fashion. Using a weekly dosing regimen, a 4-week repeat-dose toxicity study was conducted at 10, 30 and 100 mg/kg, demonstrated that BION-1301 had no effect on vital organs and other toxicology related factors examined at any dose level up to 100mg/kg. Chronic exposure to BION-1301 led to significantly reduced levels of IgA, IgG and IgM observed at all dose levels. To determine target engagement of BION-1301, quantitative assays were developed to detect free APRIL and BION-1301. BION-1301 reduced free APRIL levels in serum in a dose-dependent manner following single and multiple dose administrations. Using a MABEL approach, PK/PD modeling informed a proposed human starting dose of 50 mg. In summary, BION-1301 showed no toxicity in NHP and binding of APRIL resulted in decreased IgA, IgG and IgM levels. PK and target engagement biomarkers predicted the first-in-human dose using PK/PD pharmacometric modeling. BION-1301, which was shown preclinically to inhibit MM proliferation, survival, and drug resistance and reversed an immune suppressive phenotype, is being evaluated in previously treated MM patients in an ongoing Phase 1/2 clinical trial.

P.B1.05.05

Synergistic killing of glioblastoma stem-like cells by monoclonal antibody 8B6 specific for O-acetyl-GD2 and temozolomide

***S. Fougeray**;*
CRCINA - INSERM U1232, Nantes, France.

Introduction: Glioblastoma multiforme (GBM) is the most deadly primary brain tumors. Therapeutic options are limited and there is no cure. Moreover, almost all patient develop recurrent tumors, which are generally more aggressive. GBM recurrence is related to the presence of highly tumorigenic GBM cells, the glioblastoma stem-like cells (GSC). GSC are resistant to standard of care glioblastoma therapy consisting of radiotherapy and temozolomide (TMZ). As such, sensitizing GSC to the current standard of care will potentially improve its therapeutic impact.
Materials and methods: We first evaluated the O-acetyl-GD2 (OAcGD2) expression on GSC by flow cytometry. Next, we characterized the effects of anti-OAcGD2 monoclonal antibody 8B6 + TMZ combination on tumor cell viability, using an MTT assay and *in vivo* in an orthotopic GBM xenograft mice model.
Results: Here we found that OAcGD2 ganglioside is expressed on GSC. We further demonstrated that 8B6 + TMZ synergistically inhibited glioblastoma cell proliferation and compromised GSC survival *in vitro*. These findings correlated with longer therapeutic response evidence *in vivo*. Mechanistically, we evidenced that 8B6 oncosis-like properties increased cytotoxic drug uptake into GBM cells. As a result, 8B6 + TMZ combination induced significantly increased DNA damages and tumor cell death than either 8B6 or TMZ monotherapy.
Conclusion: Taken together, our data provides a mechanistic rationale for anti-OAcGD2 monoclonal antibodies as chemosensitizing agents to improve the response of GBM to TMZ.
Disclosure: JF, SFa and SB are designed as inventor of pending patents covering cancer immunotherapy targeting O-acetyl-GD2.

P.B1.05.06

Assessment of HER2 amplification in paired tumoural/non-tumoural gastric adenocarcinoma samples by reliable molecular biology methods

***I. Juarez**[1], J. F. Toro-Fernandez[1], A. Gutierrez[2], A. Blazquez[2], E. Ovejero[2], I. Lasa[2], A. Lopez[2], R. Gomez[2], J. M. Martin-Villa[1];*
[1]Universidad Complutense de Madrid, Dpt. of Immunology, Madrid, Spain, [2]Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Madrid, Spain.

Gastric cancer is the fifth most common malignancy, and the third cause of death by cancer in the world (survival rate below 30%). There are no specific biomarkers for this disease, and so the therapeutic approach consists of the resection of the tumour and adjuvant chemo-radiotherapy.
HER2 (Human epithelial growth factor receptor 2) is a tyrosine-kinase receptor whose genetic amplification and overexpression is a common malignant trait in breast cancer. This gives way to the treatment with Trastuzumab, a specific antibody that recognizes the HER2 malignant cells and gives patients a better predictive value. Some works have found that HER2 overexpression is not specific of breast cancer, but could also play a role in gastric adenocarcinoma.
40 samples from gastric adenocarcinoma patients were obtained at Hospital Universitario Principe de Asturias (Madrid). As positive control, DNA from cell-lines SKBR-3 and BT-474 was used.
HER2 genetic-amplification was measured by differential PCR (dPCR) for HER2 and IFN-γ, run the samples in a 3% agarose gel and calculated the bands densitometry. In addition, qPCR for the same genes was also done. In both cases, HER2-amplification was determined by measuring the HER2/IFN-γ signal ratio.
A cut-off point was established using 26 controls and confirmed HER2 amplification in 25% of gastric cancer patients by both DNA-based methods. Results were confirmed by IHC. These results suggest that HER2-positive patients are not a scarce in gastric adenocarcinoma and overexpression ought to be assessed by more than one method. For these patients, Trastuzumab might become a good therapeutic approach.

P.B1.05.07

Depletion of CAR-expressing lymphocytes using autologous anti-CAR-engrafted T cells

S. Koristka[1], *P. Ziller-Walter*[2], *A. Feldmann*[1], *C. Arndt*[1], *S. Albert*[2], *G. Ehninger*[3,4,5], *M. Bornhäuser*[3,4,5], *M. Bachmann*[1,2,4];
[1]*Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Dresden, Germany,* [2]*Tumor Immunology, UniversityCancerCenter (UCC) `Carl Gustav Carus' Technische Universität Dresden, Dresden, Germany,* [3]*Medical Clinic and Policlinic I, University Hospital `Carl Gustav Carus' Technische Universität Dresden, Dresden, Germany,* [4]*German Cancer Consortium (DKTK), partner site Dresden and German Cancer Research Center (DKFZ), Heidelberg, Germany,* [5]*National Center for Tumor Diseases (NCT), `Carl Gustav Carus' Technische Universität Dresden, Dresden, Germany.*

Adoptive transfer of chimeric antigen receptor (CAR) T cells represents one of the fastest growing areas in cancer immunotherapy. Albeit gene-modified cells have demonstrated unparalleled antitumor efficiency in B cell malignancies, highly potent CAR T cells can cause severe and partly life-threatening side effects including cytokine release syndrome, neurological toxicity and off-target effects. Hence, there is an increasing demand for developing effective approaches to selectively ablate gene-modified cells *in vivo*.
Previously, we described an epitope tag (E-tag) derived from the human nuclear protein La that is incorporated into the extracellular domain of CARs and accessible by an anti-La monoclonal antibody (mAb). Based on this mAb, we generated a novel CAR construct for specific binding and depletion of E-tag-expressing CAR T cells.
We demonstrate that anti-E-tag-redirected T cells selectively eliminate CAR T cells that extracellularly express the E-tag whilst CAR T cells lacking this tag are not attacked. Interestingly, T cell killing is reciprocal and occurs in dependence of an intracellular signaling domain. Our studies further indicate that T cells expressing high CAR levels are more efficiently depleted than T cells with low CAR expression. Besides, $CD4^+$ and $CD8^+$ target cells are equally well eliminated by both $CD4^+$ and $CD8^+$ effector T cells.
Overall, we provide an approach for specific and efficient depletion of overactive CAR T cells in case patients experience severe side effects. The E-tag can be incorporated into all CARs irrespective of the targeted tumor antigen and represents a promising tool to improve safety of cell-based immunotherapies.

P.B1.05.08

Peripheral blood immune profiling to predict response to PD-1 checkpoint blockade in patients with advanced non-small cell lung cancer

S. Lopez-Lastra[1,2], *T. Le Bourgeois*[1,3], *F. Bidard*[2,3,4], *S. Amigorena*[1,2], *O. Lantz*[1,2], *E. Romano*[1,2,3];
[1]*Center of Cancer Immunotherapy, Institut Curie, PSL Research University, Paris, France,* [2]*INSERM U932, PSL Research University, Institut Curie, Paris & St. Cloud, France,* [3]*Department of Oncology, Institut Curie, Paris & St. Cloud, France,* [4]*Université Versailles Saint-Quentin-en-Yvelines & Université Paris-Saclay, Saint Cloud, France.*

Introduction: Immune checkpoint inhibitors have remarkably improved the natural history of patients with non-small cell lung cancer (NSCLC), with improved clinical responses and overall survival compared to standard therapy. However, over 80% of unselected NSCLC patients do not respond, highlighting the need of theranostic biomarker discovery. In a cohort of NCSLC patients treated with anti-PD-1-blockade, we investigated blood immune parameters at baseline and patient characteristics as potential theranostic biomarkers. **Methods**: Thirty-four patients with locally advanced/metastatic NSCLC received either nivolumab or pembrolizumab as ≥ 2 line treatment in a prospective study at the Institut Curie. Peripheral blood mononuclear cells and serum were analyzed at baseline and correlated with outcome parameters based on immune-related RECIST criteria. **Results**: Baseline $CD3^+/CD14^+$ratio was the strongest predictive biomarker with patients achieving progression free survival (PFS) ≥4 months showing an average ratio of 1.91 vs 1.11 in patients with PFS ≤4 months (p=0,003). We found a strong positive correlation between the proportion of HLA-$DR^{hi}CD14^+$monocytes and the PFS (r=0,471), with objective responders showing higher CD86 expression, suggesting an improved antigen-presenting capacity. In addition, patients with a PFS ≥4 months, displayed higher proportions of $CCR7^-CD45RA^-$ effector memory $CD8^+$T cells and regulatory $CD4^+$T cells suggesting pre-existing adaptive immune responses. In line with previous reports, our results confirm the association of baseline serum albumin with clinical outcome in NSCLC, with levels ≥ 3.9 g/dL significantly associated with improved PFS (p=0,026).
Conclusions: Our study identifies promising, immune-related, theranostic biomarkers in NSCLC patients treated with PD-1 blockade.

P.B1.05.09

Dual CAR antiHER2-CD137 and anti-MUC1-CD3: a proposal of immunotherapy against anti-HER2 breast cancer

B. Marzal[1], *S. Betriu-Mendez*[1], *V. Ortiz-Maldonado*[2], *A. Boronat*[1], *J. Yagüe*[1], *M. Juan*[1,3], *A. Prat*[4];
[1]*Hospital Clínic - Servei d'Immunologia CDB, Barcelona, Spain,* [2]*Hospital Clínic - Servei d'Hematologia, ICMHO, Barcelona, Spain,* [3]*Plataforma d'Immunoteràpia HClínic-HSJD, Barcelona, Spain,* [4]*Hospital Clínic - Servei d'Oncologia, ICMHO, Barcelona, Spain.*

Introduction: Chimeric antigen receptors (CARs) are a new promising antitumoral immunotherapy. Despite great results in the treatment of hematologic malignancies, in solid tumors there are still some great challenges to overcome, being to avoid on-target off-tumor-effects one of the main aspects to improve. In our project we propose a novel dual CAR for the treatment of HER2+ breast cancer targeting ErbB2 (HER2) and Mucin-1 (MUC1). We present here some preclinical steps of this proposal.
Methodology and Results: After synthetizing scFv-HER2 and scFv-MUC1, we construct individuals CARs (CAR-HER2 and CAR-MUC1) with scFv sequence and 4-1BB/CD3ζ intracellular signaling domains. Delivering them into human T cells by lentiviral transduction for each CAR, we tested their specific and powerful. Dual CAR was also synthesized dissociating co-stimulatory domains: scFv-HER2 with 4-1BB and scFv-MUC1 with CD3ζ. We evaluate the specificity, functionality, and safety by comparing cytotoxicity in co-culturing with SK-BR3 and T-47D breast cancer cell lines.
Conclusions: The creation of a dual CAR to fight against breast cancer HER2+ is feasible and it is a new promising proposal for the treatment of HER2+ breast cancer tumors.
Study mainly supported by Fundación Eugenio Rodrigez Pascual PI044185 and partially by projects PI13/0676 and PIE13/033, integrated in the Plan Nacional de I+D+I (ISCIII - Subdirección General de Evaluación y Fomento de la Investigación Sanitaria - and FEDER).

P.B1.05.10

A high throughput approach for the parallel identification of TCRs recognizing multiple antigens with clinical relevance for the treatment of B cell malignancies

M. H. Meeuwsen, *C. Kweekel, L. Jahn, D. M. van der Steen, R. S. Hagedoorn, M. G. Kester, M. Griffioen, P. A. van Veelen, F. Falkenburg, M. H. Heemskerk;*
LUMC, Leiden, Netherlands.

CAR T-cell therapies for the treatment of B-cell malignancies have shown great promise in clinical trials. However, antigen negative escape variants can cause disease relapse and therefore additional therapies are needed. To increase the number of potential targets we propose a TCR-based approach to target both intracellular and extracellular proteins. Illumina HT-12 microarray data was used to select 31 target genes expressed in B-cell malignancies but not in healthy tissues other than B-cells. To broaden to scope of TCR gene therapy beyond HLA-A*02, peptides presented in HLA-A*01, A*24, B*08 or B*35 were selected. Elution of HLA-presented peptides and mass spectrometry was used, which led to the identification of 19 target genes derived peptides presented in one of the HLA-alleles of interest. Peptide-HLA tetramers were used to single cell sort tetramer+ CD8+ T-cells from healthy donor PBMCs. Donors were negative for the HLA alleles of interest to allow identification of high affinity T-cells from the allo-HLA repertoire. In total 12.300 T-cells were single-cell sorted of which 62 T-cell clones recognized one of the candidate peptides. Of these 62 T-cell clones 23 were potent enough to recognize endogenously processed and presented peptide by target gene and HLA transduced k562 cells. For safety reasons only 9 T-cell clones were selected that specifically recognized the candidate epitope without recognizing other HLA-peptide combinations. Finally 3 T-cell clones specific for two different targets effectively recognized malignant B-cells. The TCRs of these clones could be promising candidates for TCR gene transfer therapy to treat B-cell malignancies.

P.B1.05.11

z-Movi: A high throughput cell-cell avidity screening and sorting acoustic force based technology

W. Scheper[1], ***E. Merino Rodriguez***[2], *R. Braster*[2], *G. Sitters*[2], *F. Oswald*[2], *R. Driessen*[2], *T. Schumacher*[2], *A. Candelli*[2];
[1]*The Netherlands Cancer Insitute, Amsterdam, Netherlands,* [2]*LUMICKS, Amsterdam, Netherlands.*

Adoptive cell therapy can be an effective treatment option in a proportion of cancer patients. Nevertheless, the clinical efficacy of such therapies is likely limited by a lack of tools to quantitatively select and sort the most potently tumor-reactive immune cells at high throughput. Here, we developed a novel platform that uses acoustic force manipulation to query the interaction strengths of cells with their cognate binding partners. This technology, based on acoustic forces, is a lab-on-a-chip assay that allows the assessment of thousands of cell-cell interactions in parallel. Moreover, this technology provides an accurate, label-free method to isolate cells based on their avidity to specific targets, such as (tumor) cells as well as proteins, peptides, and viruses. As a proof of concept, we validated our technology by analyzing the functional avidity of T-cells towards tumor cells and found that it permits the separation of tumor-specific T-cells from non-specific bystander T-cells. These data demonstrate the potential of this platform in profiling T cell-tumor cell interaction and pave the way to quantitative cell-cell avidity studies as well as a selection of patient-specific immune effector cells or immune receptors for therapeutic use.

P.B1.05.12

HLA DP as a transmembrane anchor for chimeric TCR dimers to improve TCR transgenic therapies

L. T. Morton, *R. Hagedoorn, D. Remst, T. Jonkman, F. Falkenburg, M. H. Heemskerk;*
Leiden University Medical Centre, Leiden, Netherlands.

Ex-vivo introduction of tumour-reactive T-cell receptors (TCRs) into patient-derived T-cells result in redirected T-cell responses towards tumours. TCRαβ expression depends on formation of the CD3 signalling complex via non-covalent interactions within the transmembrane (TM) domains. Competition between endogenous TCR and introduced TCR for binding to the CD3 signalling complex, has been shown to interfere with introduced-TCR expression, thereby reducing future therapeutic efficacy. To remove competition, chimeric TCR dimers (cTD) were designed to incorporate TM domains from type I heterodimeric TM proteins, linked to the intracellular signalling domains of CD3ζ.

We hypothesised cTDs would demonstrate CD3-independent expression of introduced-TCR and allow for improved antigen-specific T-cell responses against tumour targets. Here, cTDs were engineered to incorporate 1) the extracellular domains of TCRαβ, specific for B-cell antigen BOB1, 2) the TM domains of HLA-DPαβ and 3) the intracellular CD3ζ signalling motifs (BOB1-DP-3z). Firstly, BOB1-DP-3z was expressed in $TCR^{neg}CD3^{pos}$ Jurkat-76 cell lines and demonstrated BOB1-specific pMHC-tetramer binding at the cell surface. Furthermore, in contrast to wild-type BOB1-TCR, BOB1-DP-3z expressing Jurkat-76 cells did not show surface expression of CD3, demonstrating CD3-independent expression of cTD. Next, BOB1-DP-3z was expressed in primary CD8 T-cells and co-cultured with BOB1-expressing tumour-target cells. Despite demonstrating BOB1-specific pMHC-tetramer binding, BOB1-DP-3z expressing CD8 T-cells were unable to produce functional T-cell responses against tumour targets. Inclusion of CD28 intracellular signalling motifs also did not prompt functional T-cell responses against tumour targets. In conclusion, in the context of the BOB1-specific TCR, HLA-DP appeared to be unsuitable as a TM anchor for functional TCR expression.

P.B1.05.13
Novel method for the manufacture of CAR-T-cells: Effects of different cytokines in culture media on the phenotype of CAR-T-cells

***J. Musil**, P. Ptáčková, P. Otáhal, P. Gabriel, M. Kroutilová, Š. Něměčková;*
IHBT, Prague, Czech Republic.

Current manufacture of clinical CAR-T-cells is primary based on lentiviral/retroviral transduction of CD3/CD28 activated T-cells and subsequent cultivation in the presence of IL2. This well-established approach, however has few weak points such as that it induces polyclonal T-cells activation resulting in low frequency of transduced T-cells and that the anti-CD3/CD28 activation is supra-physiological and modifies the differentiation of T-cells. Recently, alternative methods for efficient T-cells expansion were developed which are based on T-cell stimulation with antigen followed by cultivation in the presence of cytokines IL4 and IL7. We present a novel method of manufacturing CD19+ CAR-T-cell by electroporation of plasmid DNA followed by cultivation in the presence of cytokines IL2, IL4, IL7 and/or IL21 without any additional artificial T-cell activation step. This method leads to a spontaneous activation of transfected T-cells probably through recognition of endogenously present B cells, which results in overall CAR19+ cell yield (>90% CAR+) surpassing the standard methods. Cultivation of CAR19 T cells in the presence of IL4 and IL7 preferentially expands CD4+ CAR19 T-cells. Addition of IL2 or IL21 into this cytokine cocktail enhances expansion of CD8+ CAR19 T cells. However, IL21 preferentially expands CD8+ central memory T-cells. Furthermore, it enhances expression of costimulatory molecules CD28 and CD27 on CD8+ CAR-T-Cells and decreases expression of inhibitory receptors. This project is supported by grant NV15-34498A of the Ministry of Health, Czech Republic and by the European Regional Development Fund and OP RDE, Ministry of Education, Youth and Sports of the Czech Republic (project AIIHHP: CZ.02.1.01/0.0/0.0/16_025/0007428).

P.B1.05.14
Devising a dual chimeric antigen receptor system to prevent on-target off-tumor effect of engineered T cells

***T. Peters**[1], V. Gudipati[1], O. Dushek[2], M. Hudecek[3], J. B. Huppa[1];*
[1]Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria, [2]Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom, [3]Medizinische Klinik und Poliklinik II, Universitätsklinikum Würzburg, Würzburg, Germany.

CAR-T cell therapies have shown to be potent tools in the fight against cancer as validated by the recent FDA approval of two such therapies against B cell malignancies. However, a complication associated with all current CAR designs is limited tumor-specificity due to the non-existence of tumor-specific antigens for the CAR to act upon. As a consequence, CAR-T cells attack invariably healthy tissue. To overcome this impediment, we are devising a logic gate CAR-T cell system based on the combinatorial recognition of at least two antigens, which we expect to confer sufficient discriminatory power for cancer therapy with much reduced off-target toxicity. To this end we co-express in addition to the tumor-associated antigen-specific CAR a second CAR with inhibitory properties (iCAR) binding to a surface antigen exclusively present on healthy tissue to be protected from CAR-T-cell attack. For proof of concept and system optimization we derive the iCAR from the well characterized high affinity 1G4 TCR, which targets HLA-A201/NY-ESO-1 as model antigen and which can be precisely fine-tuned through the use of NY-ESO-1-derived altered peptide ligands within an affinity spectrum covering 6 orders of magnitude. Tumor-specificity, killing capacity and intracellular signaling characteristics will be assayed through (i) conventional immunological assays and (ii) a preclinical molecular imaging platform, which involves the use of protein-functionalized planar supported lipid bilayers serving as target cell surrogate in combination with advanced microscopy affording single molecule resolution. This project is funded by the Marie Sklodowska-Curie action EN-ACTI²NG program, from the European Commission.

P.B1.05.15
Increasing CAR T-cell efficacy in prostate cancer with an immunocytokine targeted to the tumour microenvironment

***E. Runbeck**, J. Maher, S. Papa;*
King's College London, London, United Kingdom.

Chimeric Antigen Receptor (CAR) T cell therapy received landmark FDA approvals for haematological indications in 2017. Solid tumours raise many challenges and anticipated breakthroughs remain elusive. Prostate cancer (PCa) is a highly prevalent disease with a significant unmet need in the advanced setting. The Prostate Specific Membrane Antigen (PSMA) CAR P28ζ has established *in vitro* and *in vivo* efficacy in PCa models but has not met expectations in clinical studies highlighting the need for strategies to enhance efficacy. We have co-expressed the chimeric cytokine receptor 4αβ (consisting of the interleukin-4 (IL-4) receptor alpha in series with the IL-2/15 receptor beta), with P28ζ (4P28ζ). In response to IL-4, 4αβ delivers an IL-2/15 signal in the CAR-T cell. Fibroblast Activation Protein (FAP) is a stromally expressed trans-membrane enzyme found in the stroma of epithelial tumours and healing wounds. We hypothesised that a FAP specific IL-4 immunocytokine would enhance 4P28ζ proliferation, survival and cytotoxicity in the tumour microenvironment. FAP-specific hybridomas were screened for FAP specificity. Single chain variable fragments (scFvs) from clones B1 and C11 were cloned into the IgG1 heavy chain expression vector with a positive control scFv ESC11 and a derivative ESC11 immunocytokine. Screening for FAP specificity was undertaken. PCa cell lines (PC3-LN3, DU145) we engineered to express PSMA, firefly luciferase and the tdTomato reporter genes. Embryonic fibroblasts (MRC5), which naturally express FAP, were transduced with the far-red mNeptune reporter gene. Co- culture ratios for PCa:MRC5 were established *in vitro* and *in vivo* for the functional testing of anti-FAP-IL4 efficacy.

P.B1.05.16
Effect of N-deglycosylation on the immunogenic and antitumor properties of hemocyanins in mammals.

***M. Salazar**[1], J. M. Jiménez[1], J. Villar[1], M. Rivera[2], M. Báez[2], A. Manubens[3], M. I. Becker[1,3];*
[1]Fundación Ciencia y Tecnología para el Desarrollo (FUCITED), Santiago, Chile, [2]Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile, [3]Biosonda S.A, Santiago, Chile.

Mollusk hemocyanins from *Concholepas concholepas* (CCH), *Fisurella latimarginata* (FLH) and *Megathura crenulata* (KLH) are glycoproteins widely used as carriers, adjuvants, and non-specific immunostimulants in cancer because they bias towards Th1 immunity. Hemocyanins are oligomeric glycoproteins (4-8 MDa), with complex didecameric structures and heterogeneous glycosylations, mainly mannose-rich N-glycans. We have demonstrated that murine antigen presenting cells incorporate hemocyanins through mannose-recognizing C-type lectin receptors (CLRs), such as Mannose Receptor (MR) and Dectin-2. However, the role of N-glycans on the immunologic properties of these proteins has not been compressively demonstrated.
We hypothesized that enzymatic N-deglycosylation of hemocyanins decreases their immunogenic and antitumor effects in mammals.
Hemocyanins were enzymatically and chemically deglycosylated, by treatment with peptide:N-glycosydase-F and sodium periodate, respectively. Biochemical analyses showed structural modifications in N-deglycosylated hemocyanins, and the presence of residual fucose-rich glycans. ELISA analyses showed a decreased binding of deglycosylated hemocyanins to chimeric receptors MR-Fc and Dectin-2-Fc. The humoral and antitumor responses were explored in the B16F10 murine melanoma model, in which mice were primed with native and N-deglycosylated hemocyanins. After 14 days, mice were challenged with melanoma cells and underwent intralesional immunotherapy. Tumor volume was higher in mice treated with N-deglycosylated KLH, compared with native KLH, while no differences were observed with N-deglycosylated CCH and FLH. The specific serum antibody titer, measured by ELISA, showed a decreased humoral response in groups immunized with N-deglycosylated hemocyanins. These results suggest that N-glycosylations of hemocyanins play a role in their structure, immunogenicity, and would contribute to their antitumor potential.
Funding: FONDECYT1151337 and FONDEQUIPEQM140151.

P.B1.05.17
Characterization of MSLN CAR T-cells in an ovarian cancer model

***E. Schoutrop**[1], I. Magalhaes[1], I. El Serafi[1], Y. Zhao[2], M. Hassan[2], J. Mattsson[1];*
[1]Karolinska Institute - Department of Oncology-Pathology, Stockholm, Sweden, [2]Karolinska Institute - Department of Laboratory Medicine, Stockholm, Sweden.

Chimeric antigen receptor (CAR) T-cells are engineered to target surface antigen with the specificity of a monoclonal antibody combined with full activation of T cell effector functions. Mesothelin (MSLN) is an attractive target for CAR T-cell immunotherapy in ovarian cancer, with 60-65% of the ovarian tumors being MSLN+. Here we compare two 2nd generation MSLN directed CAR constructs containing different co-signaling domains (MSLN-CD28z and MSLN-4-1BBz) and evaluate if MSLN CAR T-cells can effectively attack ovarian cancer *in-vitro* and *in-vivo* using an orthotopic mouse model. RD114 packaging cell lines were used for the production of γ-retroviral vectors encoding for MSLN-CD28z-CAR, MSLN-4-1BBz-CAR, MSLN, or GFP-Luciferase. Healthy donor (HD) PBMCs were transduced with MSLN-CD28z and MSLN-4-1BBz γ-retroviral vectors to generate MSLN CAR T-cells. The human ovarian cancer cell line, Ovcar-3, was used as target following transduction with MSLN and GFP-Luciferase γ-retroviral vectors.

Successful generation of MSLN-CD28z and MSLN-4-1BBz CAR T-cells from HD PBMCs was established. The newly generated MSLN-CD28z and MSLN-4-1BBz CAR T-cells showed killing capacity *in-vitro.* Stable transduction of Ovcar-3 cells with MSLN and GFP-Luciferase vector constructs was achieved. The techniques required for MSLN CAR T-cell generation were established, as were the techniques for ovarian cancer cell transduction with MSLN and GFP-Luciferase. The latter will allow for monitoring of tumor engraftment and killing by MSLN CAR T-cells *in-vivo* in the future orthotropic mouse model.

P.B1.05.18

Dual-specific T cells and an indirect vaccine eradicate large solid tumors

C. Y. Slaney[1,2], *B. von Scheidt*[1,2], *A. S. Unsworth*[1,2], *J. A. Westwood*[1,2], *A. J. Davenport*[1,2], *A. Ali*[1,2], *S. Mardiana*[1,2], *P. A. Beavis*[1,2], *D. C. Tscharke*[3], *S. A. Rosenberg*[4], *N. P. Restifo*[4], *P. Neeson*[1,2], *P. K. Darcy*[1,2], *M. H. Kershaw*[1,2];
[1]*Peter MacCallum Cancer Centre, Melbourne, Australia,* [2]*Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Australia,* [3]*The John Curtin School of Medical Research, Australian National University, Canberra, Australia,* [4]*Center for Cancer Research, National Cancer Institute, National Institute of Health, Bethesda, United States.*

While immunotherapy can eliminate substantial burdens of some leukaemias, the ultimate challenge remains the eradication of large solid tumors and metastases for most cancers. Here we generate dual-specific T cells expressing a chimeric antigen receptor (CAR) specific for Her2 and a TCR specific for the melanocyte protein (gp100). Injection of T cells, together with a vaccine that contains a recombinant vaccinia virus expressing gp100, induced durable complete remission of a variety of Her2$^+$ tumors and established metastases, some in excess of 150 mm^2, in immunocompetent mice expressing Her2 in normal tissues. Tumor destruction occurred rapidly over seven days and was associated with an extensive infiltrate of T cells. Mice that had rejected tumors were resistant to rechallenge with the same Her2$^+$ tumor cells, indicating the formation of immune memory. Furthermore, we have established methods to transduce dual-specific T cells from human peripheral blood with both a TCR specific for gp100 and a CAR for Her2. From as little as 1 ml of human buffy coat, we could generate sufficient numbers of cells for a course of treatment for a patient. The stimulation of gp100 through TCR enhanced the human dual-specific CAR T cell proliferation, secretion of IFN-γ and killing of Her2$^+$ human cancer cells *in vitro.* These characteristics were identified to be important for eradicating tumors in the mouse models. Taken together, our data provide valuable information for the development of CAR T cell therapies for patients with solid cancers and evidence for pursuing a phase I clinical trial.

P.B1.05.19

Combination therapy of CAR-NK cells and anti-PD-1 antibody displays potent efficacy against late-stage Glioblastoma and induces protective antitumor immunity

F. Strassheimer[1], *C. Zhang*[2,3], *I. C. Mildenberger*[1], *P. N. Harter*[4,3], *T. Tonn*[5], *J. P. Steinbach*[1,3], *W. S. Wels*[2,3], *M. C. Burger*[1,3];
[1]*Institute of Neurooncology, Goethe University, Frankfurt am Main, Germany,* [2]*Georg-Speyer-Haus, Institute for Tumor Biology and Experimental Therapy, Frankfurt am Main, Germany,* [3]*German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany,* [4]*Edinger Institute, Goethe University, Frankfurt am Main, Germany,* [5]*Institute for Transfusion Medicine, German Red Cross Blood Donation Service North-East and Medical Faculty Carl Gustav Carus, TU Dresden, Dresden, Germany.*

Introduction: Checkpoint inhibitors as well as adoptive cell therapy hold great promise for cancer treatment and promising treatment responses have already been demonstrated in different cancer indications. Glioblastoma (GBM) is the most common and aggressive primary brain tumor. Standard therapy prolongs life expectancy only by months. Analysis of the GBM tumor microenvironment (TME) indicates elevated suppressive leukocyte infiltration. While the surrounding brain is HER2-negative, GBM tumors are frequently HER2-positive, suggesting HER2 as a promising target for adoptive immunotherapy. Indeed, previous results show efficacy of CAR-NK cells (NK-92/5.28.z) targeted to HER2 in mouse glioma models at early stages of tumor development.
Materials and Methods: The murine glioma cell line GL261 was transfected with HER2. Tumor cells were implanted subcutaneously into C57BL/6 mice and treated either with HER2-specific NK-92/5.28.z, parental NK-92 cells, or in combination with anti-PD-1. Effects on tumor growth and survival were determined, and lymphocyte infiltration and immunosuppressive TME were characterized by flow cytometry.
Results: Combined treatment with NK-92/5.28.z cells and anti-PD-1 resulted in tumor regression and long-term survival of late-stage tumor bearing mice. Analysis of TME showed enhanced cytotoxic lymphocyte infiltration after treatment.
Conclusion: These data demonstrate enhanced efficacy of a combination of NK-92/5.28.z cells with checkpoint inhibitors in advanced tumors. Checkpoint inhibition possibly induces a cytotoxic rather than immunosuppressive TME, leading to a primed immune system. Thus, combination therapy may be a promising treatment goal for a clinical phase I/II study.

P.B1.05.20

Establishment and Application of a Panel of PBMC Humanized Mouse Tumor Models in Immuno-Oncology and Targeted Cancer Immunotherapy

L. Zhang[1], *Y. Jin*[1], *H. Wu*[1], *F. Chen*[1], *L. Zhao*[1], *X. An*[1], *W. Tan*[2], *X. Fu*[2], *M. Qiao*[1], *Q. Shi*[1], *W. Yang*[1];
[1]*Crown Bioscience, San Diego, United States,* [2]*Taicang Blood Center, Taicang, China.*

Monoclonal antibodies and checkpoint blocking approaches have achieved remarkable clinical success in cancer immunotherapy. Alongside the success of anti-PD-1 and anti-PD-L1 antibodies (such as Keytruda® and Tecentriq®), two bispecific antibodies, catumaxomab and blinatumomab have been approved to treat cancer patients, and many more bispecific antibodies are currently in preclinical or clinical development. To meet the increasing market needs for fast, reliable, and cost effective mouse tumor model systems, we have developed a panel of PBMC humanized tumor models - the MiXeno™ platform. MiXeno models can be used for a broad spectrum of applications in I/O drug discovery, including targeted cancer immunotherapy. To validate MiXeno models for targeted cancer immunotherapy, gene expression and mutation status was profiled across the CrownBio collection of over 200 xenograft models. Specific xenograft models were selected based on their tumor antigen or gene expression levels. Models which overexpressed a variety of tumor antigens (e.g. EGFR, CD47, Braf, PD-L1, etc.) were used to develop specific MiXeno tumor models via inoculation into PBMC-humanized immunocompromised mice. Reconstitution of human immune components with human PBMCs in these tumor-bearing mice provides a useful tool to evaluate targeted immunotherapeutics including bispecific T cell engagers. Graft versus host disease (GvHD) in these models can be managed by optimizing immune cell reconstitution and tumor cell engraftment. Several models from the resulting MiXeno platform have been validated using standard of care immuno-oncology drugs and characterized by immunophenotyping. Further studies are needed to expand the model collection and to extend platform applications in the I/0 space.

P.B1.06 Tumor vaccination principles and Immunotherapy - Part 6

P.B1.06.01

Influence of ionizing radiation on bispecific antibody-redirected T cells

C. Arndt[1], *D. Lindner*[2], *S. Koristka*[1], *A. Feldmann*[1], *N. Berndt*[3], *R. Bergmann*[1], *S. Albert*[2], *A. Ehninger*[4], *G. Ehninger*[5,6,3], *J. Steinbach*[1,3,6], *M. Bachmann*[1,2,3];
[1]*Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Institute of Radiopharmaceutical Cancer Research, Dresden, Germany,* [2]*UniversityCancerCenter (UCC) 'Carl Gustav Carus' TU Dresden, Tumor Immunology, Dresden, Germany,* [3]*German Cancer Consortium (DKTK), partner site Dresden; and German Cancer Research Center (DKFZ), Heidelberg, Germany,* [4]*GEMoaB Monoclonals GmbH, Dresden, Germany,* [5]*Medical Clinic and Policlinic I, University Hospital Carl Gustav Carus Dresden, Dresden, Germany,* [6]*National Center for Tumor Diseases (NCT), Dresden, 'Carl Gustav Carus' TU Dresden, Dresden, Germany.*

Introduction: Ionizing radiation not only leads to tumor cell killing via direct mechanisms, but also has positive modulating effects on the immune system. Consequently, combination of radio- and immunotherapy for treatment of cancer patients might improve clinical outcomes. Bispecific antibodies (bsAb) binding simultaneously CD3 and a tumor-associated antigen represent promising immunotherapeutic tools. As T cells are highly radiosensitive, we questioned, whether they could still be redirected via bsAb for efficient tumor cell killing after exposure to photonic radiation in context of combinatorial therapy.
Materials and Methods: By using a CD3-PSCA bsAb, the effect of γ-radiation on redirected T cells was examined on the example of prostate cancer. Therefore, T cells exposed to doses of 2-50Gy were cultured with PC3-PSCA cells in the presence or absence of the bsAb. Afterwards T cell proliferation, cytokine release and tumor cell lysis were analyzed.
Results: The CD3-PSCA bsAb engaged γ-irradiated T cells as good as unexposed T cells resulting in an efficient tumor cell lysis. However, high doses (30-50Gy) led to a slight decrease in anti-tumor cytotoxicity. Secretion of TNF, IFN-γ and IL-2 was enhanced after exposure of T cells to 2-20Gy in a bsAb-dependent manner, while proliferation and 5dsurvival was already impaired at doses ≥4Gy.
Conclusion: γ-irradiated T cells still exert a high anti-tumor reactivity upon bsAb-mediated cross-linkage. Thus, combination with radiotherapy is a feasible and promising approach. As ionizing radiation also promotes lymphocyte infiltration via indirect mechanisms, local reduction of T cell numbers due to radiation-induced cell cycle block might be compensated.

P.B1.06.02

Preclinical rationale for optimizing the tumor microenvironment for neuroblastoma immunotherapy

M. Bahri[1], *S. Vermeulen*[1], *M. Ben Mostefa Daho*[1], *S. Faraj*[1,2], *E. Thébaud*[2], *S. Fougeray*[1], *S. Birklé*[1];
[1]*CRCINA, INSERM, Angers University, Nantes University, Nantes, France,* [2]*CHU of Nantes, Nantes, France.*

Introduction: Immunotherapy targeting GD2 in combination with IL-2 and isotretinoïn has emerged as a promising intervention for patients with neuroblastoma. The primary anti-GD2 antibody mechanism of action is based on antibody-dependent cell cytotoxicity induced by NK cells. However, a third of patient presents immunotherapy failure. One reason is the tumor microenvironment, which potentially inhibits optimal anti-tumor response. In this context, elucidation of the potential mechanisms of response merits consideration. Here we evidence by which isotretinoïn induces tumor immunotherapy escape.
Methods: We evaluated the anti-neuroblastoma response using isotretinoïn and anti-O-acetyl GD2 antibody 8B6 in the syngeneic NXS2 neuroblastoma model. Next, we studied the immune modulation induced by isotretinoïn. To this end, we studied the immune cell infiltrate composition and the immune checkpoint inhibitor PD1-L expression in NXS2 tumor in each treatment group by flow cytometry. Results: Treatment with isotretinoïn + 8B6 antibody outperformed treatments with either isotretinoïn or 8B6 antibody used as monotherapy. This effect was correlated with an increased percentage of NK cells in the tumor compared to controls. Isotretinoïn treatment also up-regulated the expression of PD1-L immune checkpoint inhibitor on the tumor cells.
Conclusion: In the isotretinoïn + 8B6 treated mice, the increase of tumor-infiltrating NK cells could contribute to potent 8B6 therapeutic efficacy. However, a better understanding of the cellular and the molecular changes following isotretinoïn is necessary to understand resistance mechanisms to anti-ganglioside immunotherapy in patients with neuroblastoma.
Disclosure: SFa and SB are designed as inventor of pending patents covering cancer immunotherapy targeting O-acetyl-GD2.

P.B1.06.03

Retargeting T-cell cytotoxicity to a unique sialylated epitope on CD43 present in acute myeloid leukemia and melanoma

L. Bartels[1], *G. de Jong*[1], *M. Gillissen*[1], *E. Yasuda*[2], *V. Kattler*[2], *C. Bru*[2], *C. Fatmawati*[2], *A. Bakker*[2], *P. M. van Helden*[2], *J. Villaudy*[2], *M. D. Hazenberg*[3], *H. Spits*[1], *K. Wagner*[2];
[1]*AIMM Therapeutics & Academic Medical Center, Amsterdam, Netherlands,* [2]*AIMM Therapeutics, Amsterdam, Netherlands,* [3]*Academic Medical Center, Amsterdam, Netherlands.*

Introduction: From the B cells of an acute myeloid leukemia (AML) patient in long-term remission, we obtained the antibody AT1413. AT1413 binds a unique sialylated epitope on CD43 present in AML and other cancers, CD43s, and may be an interesting candidate for a bispecific T-cell engaging antibody (bTCE).
bTCEs are powerful, clinically validated tools to induce T-cell cytotoxicity independent of the T-cell receptor specificity by simultaneously binding a cancer surface antigen and CD3ε.
Methods: Using site-specific, chemo-enzymatic linkage, we attached an antibody fragment targeting CD3ε to an AT1413 variant unable to interact with Fc-receptors to generate AT1413 bTCE.
The capacity of AT1413 bTCE to induce tumor cell lysis was assessed in a T-cell cytotoxicity assay; T-cell activation marker upregulation and proliferation were monitored. AT1413 bTCE was tested *in vivo,* in two mouse models, one co-injected with human PBMC, the other engrafted with a human immune system (HIS) at birth.
Results: *In vitro*, AT1413 bTCE potently induced T-cell mediated lysis of different CD43s-expressing AML cell lines, primary AML blasts and melanoma cells. Endothelial cells with detectable, but low binding of AT1413 remained unaffected. T-cell activation and proliferation were observed only in the presence of target-expressing cells. I*n vivo* testing of AT1413 bTCE (2 mg/kg, biweekly) revealed potent AML tumor growth inhibition of 89-99 % compared with a control bTCE. In the HIS-model, normal human hematopoietic cells remained present in AT1413 bTCE treated mice.
Conclusion: Our results suggest that AT1413 bTCE, which recruits T cells to CD43s-expressing tumor cells, has therapeutic potential.

P.B1.06.04

Tumor eradication through CD47 blockage and immune response induction using cancer targeted immunogene therapy

M. Billerhart[1], *M. Schönhofer*[1], *S. Eckmann*[1], *A. Kassem*[1], *W. Polzer*[1], *M. Anton*[2], *J. Maier*[1], *A. Taschauer*[1], *H. Sami*[1], *M. Ogris*[1];
[1]*Laboratory of MacroMolecular Cancer Therapeutics (MMCT), Department of Pharmaceutical Chemistry, Centre of Pharmaceutical Sciences, University of Vienna, Vienna, Austria,* [2]*Institute of Experimental Oncology and Therapy Research and Institute of Molecular Immunology, Klinikum rechts der Isar, Technische Universität München, Munich, Germany.*

The overexpression of CD47 correlates with dismal prognosis in a broad range of cancers, since the interaction of CD47 with its ligand SIRPα (on macrophages and dendritic cells) prevents cancer cell eradication. Antibodies have been evaluated in advanced clinical trials for both blocking CD47 and triggering of antibody directed cytotoxicity. Unfortunately, their systemic application causes side effects like anemia, as CD47 is also abundantly expressed on non-malignant cells including erythrocytes.
Our approach of cancer targeted immunogene therapy circumvents these side effects by triggering the expression of CD47 blocking proteins within tumor cells.
We have cloned a plasmid vector encoding for the secreted fusion protein SIRPα-Fc consisting of the high affinity CD47 binding protein CV1 fused to a human IgG1 Fc part. After transient transfection of tumor cell lines we could demonstrate secretion of SIRPα-Fc, CD47 binding and -blockage on CD47 overexpressing cells. A potent bystander effect was demonstrated when transferring SIRPα-Fc containing supernatant from transfected cells to CD47 positive recipient tumor cells.
SIRPα-Fc transfected and luciferase-marked human MDA-MB-231 triple negative breast cancer cells were implanted orthotopically into SCID mice and tumor growth rate was evaluated by bioluminescence imaging (BLI). Here, the BLI tumor cell signal of SIRPα-Fc transfected cells strongly decreased when compared to controls.
In summary, we could demonstrate that the tumor-restricted secretion of the high affinity CD47 binder SIRPα-Fc is a potential gene therapy approach to prevent side effects in non-target organs, while unleashing the full antitumoral potential of CD47 blockade and immune activation.

P.B1.06.05

Siglec-15 is a rapidly internalized cell-surface antigen expressed by acute myeloid leukemia cells

H. Cao[1], *A. Neerincx*[2], *B. de Bono*[3], *U. Lakner*[4], *C. Huntington*[5], *J. Elvin*[5], *E. Gudgin*[6], *C. Pridans*[6], *B. Huntly*[6], *J. Trowsdale*[2], *A. D. Barrows*[7];
[1]*Institute of Medical Sciences, Aberdeen, United Kingdom,* [2]*Immunology Division, Pathology Department, University of Cambridge, Cambridge, United Kingdom,* [3]*Auckland Bioengineering Institute, University of Auckland, Auckland, New Zealand,* [4]*Medical Faculty, University of Tuebingen, Tuebingen, Germany,* [5]*MedImmune, Milstein Building, Granta Park, Cambridge, United Kingdom,* [6]*Cambridge Institute for Medical Research, University of Cambridge, Cambridge, United Kingdom,* [7]*Department of Pathology and Immunology, Washington University School of Medicine, St Louis, United States.*

Acute Myeloid Leukemia (AML) is a clinically heterogeneous disease that is difficult to treat due to the lack of consistently expressed specific target cell antigens. Toxin-conjugated anti-Siglec-3 (also known as CD33) antibody may provide a useful therapeutic tool. However, CD33 is not expressed by all AML cells. Siglec-15 can be distinguished from other Siglec family members by its divergent amino acid sequence, genomic location and evolutionary conservation from mammals to fish. We raised a monoclonal antibody, A9E8, specific for Siglec-15 using phage display. A9E8 detected cell-surface expression of Siglec-15 protein on leukemic cell lines of the myeloid lineage as well as on donor blasts from AML patients. Sub-populations of $CD33^+$ and $CD33^-$ blasts from nine of twelve AML donors exhibited high cell-surface Siglec-15 expression. In contrast, there was minimal expression of Siglec-15 on the surface of peripheral blood leukocytes from healthy donors, suggesting Siglec-15 may have significant therapeutic advantage over targeting CD33. Upon binding to Siglec-15, A9E8 was rapidly internalized ($t_{1/2}$, 180sec) from the cell-surface of K562 cells. Therefore, antibodies to Siglec-15 may hold therapeutic potential for a subset of $CD33^+$ and $CD33^-$ AMLs.

P.B1.06.06

DC-SIGN is an uptake receptor for melanoma-derived autophagosomes in human dendritic cells

T. T. H. Eisden[1,2], *C. M. Ribeiro*[2], *R. Van de Ven*[1], *T. D. de Gruijl*[1], *T. B. Geijtenbeek*[2];
[1]*Department Medical Oncology, VU University Medical Center, Amsterdam, Netherlands,* [2]*Department of Experimental Immunology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands.*

Cross-presentation of tumor-associated antigens (TAAs) by dendritic cells (DCs) is critical for the induction of anti-melanoma cytotoxic T cells. However, melanoma-derived TAAs contribute to an immune suppressed tumor microenvironment and thereby halt DC maturation. We hypothesize that melanoma-derived autophagosomes contain a broader spectrum of TAAs as well as damage associated molecular patterns that could potentially break tolerance and induce anti-tumor T-cell responses.
We isolated autophagosomes from the human melanoma cell line SK-MEL-28 by mild sonication followed by differential centrifugation. Immunoblotting analysis showed that the isolated fraction was positive for the autophagosomal markers LC3-II, Atg5 and Atg16L1. Furthermore, high-resolution flow cytometry analysis confirmed enrichment for LC3-II positive vesicles. To determine the autophagosome binding receptors, isolated autophagosomes were labelled with a fluorescent dye and subsequently targeted to monocyte-derived DCs. Notably, blocking experiments showed that the C-type lectin receptor DC-SIGN is involved in the binding and uptake of the autophagosomes by DCs. Binding experiments in DC-SIGN-transfectants corroborated the role of DC-SIGN as an autophagosome targeting receptor. Furthermore, DCs incubated with autophagosomes upregulated the maturation markers CD80, CD86, CD40 and CD70, which suggest that melanoma-derived autophagosomes are immunogenic. In addition, our preliminary data show that these autophagosomes may have the potential to evoke an anti-melanoma cytotoxic T cell response.

P.B1.06.07

Redirection of switchable UniCAR T cells against radioresistant cancer cells

A. Feldmann[1], *C. Arndt*[1], *R. Bergmann*[1], *N. Berndt*[2], *J. Jureczek*[2], *S. Albert*[3], *D. Lindner*[3], *S. Koristka*[1], *J. Steinbach*[1,2,4], *G. Ehninger*[2,4,5], *M. Krause*[2,6,7], *I. Kurth*[6], *A. Dubrovska*[2,6,7], *M. Bachmann*[1,2,3];
[1]*Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Institute of Radiopharmaceutical Cancer Research, Dresden, Germany,* [2]*German Cancer Consortium (DKTK), partner site Dresden; and German Cancer Research Center (DKFZ), Heidelberg, Germany,* [3]*UniversityCancerCenter (UCC) 'Carl Gustav Carus' TU Dresden, Tumor Immunology, Dresden, Germany,* [4]*National Center for Tumor Diseases (NCT), Dresden, 'Carl Gustav Carus' TU Dresden, Dresden, Germany,* [5]*Medical Clinic and Policlinic I, University Hospital Carl Gustav Carus Dresden, Dresden, Germany,* [6]*OncoRay-National Center for Radiation Research in Oncology, Medical Faculty and University Hospital Carl Gustav Carus, TU Dresden, Dresden, Germany,* [7]*Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiooncology-OncoRay, Dresden, Germany.*

Introduction: Radiation is a common therapy for solid tumors. Unfortunately there is a high risk for the outgrowth of radioresistant tumor cells against which only limited treatment options exist. We challenged the idea whether or not chimeric antigen receptor (CAR) engineered T lymphocytes could be used as an adjuvant immunotherapy in combination with radiotherapy. Recently, we have established switchable universal CARs (UniCARs) that bind to a short peptide epitope (E5B9) which does not exist on the surface of living cells. UniCAR T cells are exclusively redirected to tumor cells in the presence of a target module (TM) that exhibits the E5B9 epitope and binds to a tumor associated antigen (TAA) on the tumor cell surface.
Materials and Methods: We used different radioresistant sublines of the head and neck cancer cell line Cal33. Gene expression data for certain TAAs were confirmed by flow cytometry. TMs against potential targets were created from the variable domains of monoclonal antibodies, cloned in lentiviral vectors and purified from supernatants of permanently TM producing 3T3 cells. UniCAR T cells were generated by lentiviral transduction.
Results: Radioresistant Cal33 cell lines expressed PSCA, EGFR and CD98. UniCAR TMs were created against these TAAs. Armed with these TMs UniCAR T cells efficiently killed radioresistant Cal33 cells *in vitro* and *in vivo*.
Conclusions: Radioresistant tumor cells can efficiently be killed by redirecting UniCAR T cells against PSCA, CD98 and EGFR and thus resistance to radiotherapy can be overcome by immunotherapy based on the UniCAR technology to these targets.

P.B1.06.08

Utilising TAPBPR to increase tumour immunogenicity

T. Ilca*, A. Neerincx, M. Wills, M. de la Roche, L. H. Boyle;*
University of Cambridge, Cambridge, United Kingdom.

As the best clinical responses to immunotherapy are often observed in patients with highest mutational burden and neoantigen presentation, the ability to boost presentation of immunogenic peptides on major histocompatibility complex class I (MHC I) molecules has the potential to improve immunotherapy efficacy. We have recently revealed that the tapasin-related molecule TAPBPR functions as an MHC class I peptide exchange catalyst. Although TAPBPR usually resides intracellularly, here we explored whether extracellular TAPBPR can be utilised to enhance the loading of immunogenic peptides on MHC class I molecules expressed on the surface of cells. We reveal that both plasma membrane-expressed TAPBPR as well as its ectodomain alone, added exogenously to cells, can function as peptide exchange catalysts on surface MHC I molecules. We can utilise these two forms of TAPBPR to display both tumour antigens and viral peptides on human MHC I molecules expressed on tumour cells and consequently induce T cell receptor engagement and killing of tumours by cytotoxic T lymphocytes. Our findings highlight a potential therapeutic application of TAPBPR in increasing the immunogenicity of tumours.

P.B1.06.09

Do IgG1 or IgG4 subclasses differently affect ADCC and ADCP against EGFR+ tumor cells?

S. A. F. Jensen[1,2], *G. Jordakieva*[2,3], *M. Bergmann*[4], *J. Laengle*[4], *S. N. Karagiannis*[5,6], *E. Jensen-Jarolim*[1,2], *R. Bianchini*[1];
[1]*Comparative Medicine, The Interuniversity Messerli Research Institute of the University of Veterinary Medicine Vienna, Medical University Vienna and University Vienna, Vienna, Austria,* [2]*Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria,* [3]*Department of Physical Medicine, Rehabilitation and Occupational Medicine, Vienna, Austria,* [4]*Division of General Surgery, Department of Surgery, Comprehensive Cancer Center Vienna, Medical University of Vienna, Vienna, Austria,* [5]*St. John's Institute of Dermatology, School of Basic & Medical Biosciences, King's College London & NIHR Biomedical Research Centre at Guy's and St. Thomas' Hospital and King's College London, Guy's Hospital, King's College London, London, United Kingdom,* [6]*Breast Cancer Now Research Unit, School of Cancer & Pharmaceutical Sciences, King's College London, Guy's Cancer Centre, London, United Kingdom.*

Background: Monoclonal anti-tumor antibody treatments could be considered as one of the most successful therapeutic strategies. Their major mechanism of action is the induction of an antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP). Our previous *in vitro* studies showed that IgG4 can drive M2a macrophages to an immunoregulatory phenotype. In the light of these data we hypothesized that IgG1 and IgG4 isotypes may differentially polarize tumor-associated macrophages (TAM), thereby impacting the quality of ADCC and ADCP against tumor cells. Methods: *In vitro*, monocyte-derived macrophages (MDMs) or monocyte differentiated cell line (U937) were treated with M-CSF and IL-4/IL-13 (M2a) to induce a M2-like polarization. A431 and Caco-2 tumor cell lines were used to test the anti-EGFR IgG1 or IgG4 mediated ADCC and ADCP with polarized U937 or MDM as effector cells. The analyses were performed by FACS and microscope immunofluorescence. Results: Our preliminary data demonstrate that treatment with IgG1 or IgG4 anti-EGFR influenced ADCC and ADCP activities by macrophages, resulting in a modulation of the killing effect of high EGFR expressing A431 cells compared to low EGFR expression Caco-2. Conclusion: Our results indicate that the specific use of IgG1 or IgG4 monoclonal anti-tumor antibody treatments may critically influence treatment outcome by modulating ADCC and ADCP mediated macrophage response. We propose that the understanding of interaction of specific anti-tumor IgG subclasses with Fc-gamma receptors expressed by TAM will help to better predict the outcome of anti-cancer immunotherapies. Supported in part by Austrian Science Fund FWF projects SFB F4606-B28 to EJJ

P.B1.06.10

Preclinical development and production, and preliminary data of the first phase I fully academic European clinical trial with an anti-CD19 chimeric antigen receptor for B-cell leukaemia and lymphoma.

M. Juan[1,2,3], *M. Castellà*[3], *M. Caballero*[1,2], *A. Boronat*[3], *V. Ortiz-Maldonado*[4], *V. Rodriguez*[3], *G. Suñé*[3], *R. Martín-Ibáñez*[5], *J. Tabera*[6], *B. Marzal*[7], *J. Castaño*[8], *E. Gonzalez-Navarro*[1], *C. Bueno*[8], *O. Balague*[1], *C. Serra-Pages*[1], *D. Benitez*[1], *P. Engel*[5], *J. Cid*[1], *A. Vilarrodona*[6], *R. Vilella*[1], *P. Menéndez*[8], *M. Lozano, T. Baumann, J. Esteve, E. Campo,*[1], *E. Trias*[6], *J. M. Canals*[5], *A. Urbano-Ispizua, J. Yague, S. Rives, J. Delgado*[1];
[1]*Hospital Clínic - Universitat de Barcelona, Barcelona, Spain,* [2]*Hospital Sant Joan de Deu, Plataforma Inmunoterapia, Unitat Funcional Immunologia Clínica, Barceliona, Spain,* [3]*IDIBAPS, Barcelona, Spain,* [4]*Hospital Clínic, Barcelona, Spain,* [5]*Universitat de Barcelona, Barcelona, Spain,* [6]*Hospital Clínic - Banc de Sang i Teixits, Barcelona, Spain,* [7]*Fundació Clínic, Barcelona, Spain,* [8]*Institut Josep Carreras, Barcelona, Spain.*

Chimeric antigen receptor (CAR) have shown impressive treating responses against CD19+ B-lymphoproliferative malignancies. To make available this treatment to our patients, we develop our "own CART19-cells" (ARI-001) based on A3B1 anti-CD19 antibody, followed by CD8 hinge/transmembrane region, 4-1BB and CD3z signaling domains. Preclinical studies [*in vitro* we show that ARI-0001 were highly active (cytotoxicity, secretion of cytokines and proliferation) + *in mouse*, ARI-0001 were able to control disease progression in a NSG xenograph B-ALL mice. After reaching all pre-specified acceptance criteria, the Spanish Agency of Medicines approved our IND and also our first pilot clinical trial (*NCT03144583; EudraCT: 2016-002972-29*) on May/2017, being the first phase-I fully academic European clinical trial with CARTs. Until now (April 2018), we have recruited 21 patients with: 17 patients with acute lymphoblastic leukemia (B-ALL), 3 patients with Non-Hodgkin B-cell lymphoma and 1 patient with chronic lymphocytic leukemia. We successfully prepared ARI-0001 cells in all patients, although 3 patients required two procedures (3/24 [12.5%] production failure). After 8-10 days of culture, 1,275 x10^6 cells (range, 230-2500x10^6) were obtained as median value. The median percentage of ARI-0001 in infusible product was 28.85% (range, 20.4-72.3). After patient's conditioning, we infused 0.5-5x10^6 ARI-0001/kg to 17 patients. In all infused patients, ARI-0001 cells were observed in peripheral blood together with B-cell aplasia; no uncontrolled severe events were obtained. Preliminary data about efficacy show levels of response similar to other CART19s currently being used in clinic. *Authors were supported in their works by projects PI13/00676, PICI14/00122 and PIE/00033*

P.B1.06.11

MISTRG: improved human immune system mouse model to test transplantable T cell therapy against solid tumors

J. J. Karrich[1], *R. de Groot*[1], *K. J. Hartemink*[2], *J. B. Haanen*[2], *M. C. Wolkers*[1], *D. Amsen*[1];
[1]*Sanquin Reserch, Amsterdam, Netherlands,* [2]*Netherlands Cancer Institute, Amsterdam, Netherlands.*

Transfusion of *in-vitro* expanded T cells isolated from tumors (Tumor-Infiltrating lymphocytes or TILs), back into patients is a promising form of therapy for yet untreatable types of cancer, such as melanoma. Although significant progress has been achieved in optimizing in-vitro TIL cultures, many parameters affecting T cell responses against tumors cannot be approximated *in-vitro*. Therefore, we aim to establish and characterize a human immune system mouse model to study TIL responses to tumors in vivo. To this end, we are using the newly developed "MISTRG" mouse model, which allows superior development of a human immune system, which includes both lymphocytes as well as functional myeloid and NK cells at close to physiological levels. Importantly, these mice allow establishment of human tumors that are histologically similar to human tumors in terms of vascularization and infiltration of myeloid cells.

This model therefore more reliably mimics human tumors than traditional models using NSG mice. **We will use this new model to engraft patient-derived non-small cell lung carcinoma (NSCLC) tumor cells and subsequently test *ex-vivo* expanded TILs in an autologous setup.** To do so, tumor cells are isolated isolated directly from patient biopsies and expanded *ex-vivo* as organoid culture, cell line culture, or *in-vivo* in a xenograft model. We will use this model to study parameters that determine the outcome of TIL therapy and to develop and test different treatment modalities.

P.B1.06.12

Minimal residual disease monitoring in course of immunotherapy of acute lymphoblastic leukemia

A. Y. Komkov[1,2], *A. M. Miroshnichenkova*[2], *G. A. Nugmanov*[1], *Y. B. Lebedev*[1], *M. A. Maschan*[2], *Y. V. Olshanskaya*[2], *I. Z. Mamedov*[1];
[1]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russian Federation,* [2]*Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation.*

Anti-CD19 immunotherapy based on CAR-T and bispecific antibodies significantly increased the efficiency of refractory leukemia treatment. However, it also brought a new challenge such as increasing frequency of CD19-negative relapses including lineage differentiation switching. Minimal residual disease (MRD) diagnostics is the tool which allow to reveal the risk of relapse by leukemic cells concentration measurement right after therapy. The most common method for MRD diagnostics is flow cytometry which, however, have significant limitations due to target surface markers loss during immunotherapy. Here we present marker expression independent method for MRD monitoring based on high-throughput sequencing (HTS) of clonal rearrangements of T- and B-cell receptors loci.
Leukemia-specific rearrangements were identified by analysis of their frequencies in sequenced amplicons obtained in multiplex PCRs with primers for vast majority of rearranged TCR and BCR loci. MRD monitoring was performed by HTS-based detection of previously identified rearrangements in samples after therapy and subsequent quantitative analysis using statistical principal of digital PCR.
The developed method was tested on cohort of 20 patients received standard chemotherapy. Over 100 clonal rearrangements were detected in initial samples in total. All identified rearrangements were used for subsequent MRD monitoring. Obtained MRD results were highly concordant with ones detected by flow cytometry. The pilot MRD monitoring by developed method after immunotherapy was performed for four patients. All these cases were MRD negative.
Being highly accurate and sensitive, HTS of rearranged TCR/BCR loci is most promising alternative of flow cytometry for MRD diagnostics in leukemia immunotherapy. Funds: RSF grants №17-75-10113, №18-14-00244.

P.B1.06.13

Development of a GMP-compliant two-step maturation process for the generation of plasmacytoid dendritic cells as anti-tumor vaccine

L. Kurz[1], *N. Smith*[2], *T. Fecher*[1], *E. Dimitriou*[1], *T. Trzaska*[1], *A. Felsen*[1], *P. Reinhardt*[1,3], *P. Schuler*[4], *J. Münch*[2], *T. Hoffmann*[4], *H. Schrezenmeier*[1,3], *D. Fabricius*[5], *B. Jahrsdörfer*[1,3];
[1]*Department of Transfusion Medicine, Ulm, Germany,* [2]*Institute for Molecular Virology, Ulm, Germany,* [3]*Institute for Clinical Transfusion Medicine and Immunogenetics, German Red Cross Blood Transfusion Service Baden-Württemberg – Hessen and University Hospital Ulm, Ulm, Germany,* [4]*Department of Otolaryngology, Ulm, Germany,* [5]*Department of Pediatrics, Ulm, Germany.*

Allogeneic plasmacytoid dendritic cells (pDC) from partly HLA-matched healthy donors may represent a promising alternative to conventional DC as anti-tumor vaccine. Recently, GMP-compliant isolation of pDC precursors from peripheral blood became possible, so that their clinical application appears within reach. In our current study, we tested a GMP-compliant positive selection kit to isolate BDCA-4$^+$ pDC from leukapheresis products collected from 26 unstimulated healthy donors. After isolation, pDC precursors can be activated and matured within 24-48 hours. On average, we retrieved 4.4x10^6 pDC from ~1.9x10^9 total PBMC. Purity was 95.9%, viability 94.5%. Extrapolated to the size of a full leukapheresis product, it is possible to isolate >20x10^6 viable pDC precursors. After pulsing pDC with K562 lysate, followed by TLR-stimulation, we tested the capacity of pDC to generate cytotoxic T cells via CFSE staining, intracellular IFN-γ staining, and a Europium-based cytotoxicity assay. We observed that maturation of pDC consists of a biphasic activation process. During phase 1, immature pDC expressed high levels of proteases and few costimulatory molecules. Confocal microscopy demonstrated that only in this phase pDC can take up antigens from K562 lysates. In phase 2, antigen-loaded pDC matured by TLR ligands downmodulated proteases, but upregulated costimulatory molecules and MHC/peptide complexes. Mature pDC induced IFN-γ expression and proliferation of allogeneic CD8$^+$ cytotoxic T cells. Moreover, after expansion in the presence of K562 lysate-pulsed pDC, cytotoxic T cells were able to kill K562 cells. Our study indicates that GMP-compliant generation of pDC as allogeneic anti-tumor vaccine is feasible.

P.B1.06.14

Evaluation of TfR1 as a suitable receptor for intracellular drug delivery via an antibody drug conjugate.

A. Laroche[1], *R. Melhem*[1], *M. Pugnière*[1], *C. Martin*[2], *N. Joubert*[2], *C. Larbouret*[1], *A. Pèlegrin*[1], *M. Poul*[1];
[1]*IRCM - U1194, Montpellier, France,* [2]*GICC CNRS UMR. 7292, Tours, France.*

Transferrin receptor 1 (TfR1) is a cell surface receptor overexpressed in various cancers. TfR1 provides iron to cells for their metabolic activity by mediating the internalization of iron loaded transferrin (holo-Tf). Our lab has recently generated an internalizing anti-TfR1 antibody (H7) that blocks holo-Tf internalization and deprives efficiently cancer cells from iron. H7 treatment led to cell death in lymphoma and leukemia cell models and to S-phase blockade in pancreatic adenocarcinoma (PDAC) cells models, *in vitro*. To enhance its toxic activity, H7 was now conjugated to the microtubule inhibitor monomethyl auristatin F (MMAF).
Methods: The anti-TfR1 H7 was conjugated to auristatin F with a non-cleavable linker to obtain an antibody drug conjugate (ADC). Negative controls anti-CD20 rituximab was obtained the same way. The anti-CD30 ADC brentuximab vedotin was used as a positive control for its activity on Karpas lymphoma cell line. ADC activity was measured by MTS assay.
Results: H7-MMAF was more potent at reducing CD30 positive lymphoma Karpas cell line viability *in vitro* than the reference anti-CD30 ADC brentuximab vedotin. (2H7-MMAF highly reduced BxPC3 and CFPAC PDAC cell lines viability *in vitro* compared to the naked antibody H7. These data suggest that TfR1 is suitable receptor for microtubule inhibitor intracellular delivery in cancer. H7-ADC iron deprivation intrinsic activity likely combines to microtubule inhibitor to kill cancer cells. Toxicity assessment of H7-MMAF on normal cells will be crucial for further development.

P.B1.06.15

CD20 and CD37 antibodies cooperatively induce killing of malignant B-cells through complement-dependent cytotoxicity

S. C. Oostindie[1,2], *H. van der Horst*[3], *M. A. Lindorfer*[4], *E. M. Cook*[4], *C. S. Zent*[5], *M. E. Chamuleau*[3], *E. C. Breij*[3], *T. Mutis*[3], *F. J. Beurskens*[1], *P. W. Parren*[2], *R. P. Taylor*[4];
[1]*Genmab, Utrecht, Netherlands,* [2]*Leiden University Medical Center, Leiden, Netherlands,* [3]*VU University Medical Center, Amsterdam, Netherlands,* [4]*University of Virginia School of Medicine, Charlottesville, United States,* [5]*University of Rochester Medical Center, New York, United States.*

In recent years, B-cell malignancies have been successfully targeted with anti-CD20 monoclonal antibodies (mAbs). Another interesting B-cell target is CD37, targeting of which by therapeutic antibodies is currently undergoing clinical evaluation. While known CD20 antibodies can employ complement-dependent cytotoxicity (CDC) as an efficient effector mechanism, currently known CD37 antibodies are poor inducers of CDC. Antibody engineering and drug combination studies are promising strategies to enhance antibody mediated effector functions. For example, CDC efficacy can be improved by introducing single point mutations in the Fc domain that enhance intermolecular Fc-Fc interactions between IgG molecules after cell surface antigen binding, thereby facilitating IgG hexamer formation. In this study, we explored whether introducing such a hexamerization-enhancing mutation into CD37-targeting mAbs could potentiate CDC in malignant B-cells, and whether CDC could further be enhanced by combinations of CD37 and CD20 mAbs. Interestingly, introducing a hexamerization-enhancing mutation into CD37 mAbs resulted in enhanced CDC of tumor B-cells. More striking, combinations of hexamerization-enhanced CD20 and CD37 mAbs showed superior CDC *ex vivo* in tumor cells obtained from patients with B-cell malignancies, compared to the single agents alone. Furthermore, in depth analysis into the mechanism behind enhanced CDC activity demonstrated that, upon target binding, CD20 and CD37 antibodies co-localized on the cell surface and substantially enhanced C1q binding and recruitment, indicating more efficient activation of complement components. These results provide a rationale for antibody combinations to enhance CDC, and provide new mechanistic insights into cooperative interactions between antibody molecules leading to highly efficient CDC activity.

P.B1.06.16

Efficacy of ex-vivo PD-1 blockade in cervical tumor-draining lymph nodes is related to a CD8$^+$ T-cell subset with high levels of immune checkpoints and superior poly-functional effector functions

J. Rotman[1,2], *A. M. Heeren*[1,2], *A. S. Stam*[1,3], *N. Pocorni*[1], *C. H. Mom*[4,2], *M. C. G. Bleeker*[5], *J. van der Velden*[4,2], *H. J. Zijlmans*[6,2], *G. G. Kenter*[2], *E. S. Jordanova*[1,2], *T. D. de Gruijl*[1,3];
[1]*Cancer Center Amsterdam, Amsterdam, Netherlands,* [2]*Center for Gynecologic Oncology Amsterdam (CGOA), Amsterdam, Netherlands,* [3]*Department of Medical Oncology, VUmc, Amsterdam, Netherlands,* [4]*Academic Medical Center (AMC), Amsterdam, Netherlands,* [5]*Department of Pathology, VUmc, Amsterdam, Netherlands,* [6]*Netherlands Cancer Institute, Amsterdam, Netherlands.*

Introduction: An important prognostic factor in cervical cancer (CxCa) is lymph node metastasis. High and interrelated rates of Tregs and PD-L1-positive macrophages in metastatic tumor-draining lymph nodes (TDLN) previously pointed to the applicability of PD-(L)1 blockade to halt metastatic spread. Here, we show the ex-vivo efficacy of PD-1 blockade in CxCa primary tumors (PT) and TDLN and relate it to the presence of a specific CD8$^+$ effector T-cell subset.
Materials & Methods: The effect of anti-PD-1 on T-cell reactivity against the HPV16 E6 oncoprotein in TDLN (n=12) and PT (n=7) single cell suspensions was assessed after 10 days in-vitro culture by IFNγ Elispot read-out. Multicolor flowcytometric analysis of T-cells in TDLN (n=23) and PT (n=10) was also performed.

Results: Consistently enhanced T-cell responses to HPV16 E6 upon PD-1 blockade were observed in all tested metastatic TDLN, but remarkably only in 1/4 HPV16$^+$ PT. Extensive flowcytometric analysis revealed a selective and significant correlation between the ex-vivo efficacy of PD-1 blockade and frequencies of CD8$^+$CD25$^+$FoxP3$^+$ T-cells. This subset was characterized by an effector phenotype with elevated expression levels of PD-1, CTLA4, TIM3 and LAG3 checkpoints, but, rather than exhausted, was shown upon activation to express higher levels of Granzyme-B and effector cytokines as compared to its CD8$^+$FoxP3- counterparts.
Conclusion: These data support the earlier reports of a "poised" HPV-specific T-cell repertoire in TDLN and show them to be valid targets for PD-1 blockade. Moreover, they point to CD8$^+$CD25$^+$FoxP3$^+$ T-cells as likely therapeutic target, which may also serve as predictive biomarker.

P.B1.06.17
Modular design of a trispecific T-cell engager antibody (TriTE) for dual targeting of colorectal cancer

*A. Tapia[1], R. Navarro[1], M. Compte[1], A. Erce[1], M. Zonca[1], L. Alvarez-Vallina[2], **L. Sanz**[1];*
[1]Molecular Immunology Unit, Hospital Puerta de Hierro, Majadahonda, Spain, [2]Immunotherapy and Cell Engineering Laboratory, Department of Engineering, Aarhus University, Aarhus, Denmark.

Introduction: *Biespecific T cell Engager*s (BiTE) are engineered antibody constructs composed of an anti-CD3 single-chain variable fragment (scFv) linked to an anti-tumor-associated antigen (TAA) scFv. To minimize the risk of "on-target/off-tumor" effects, we propose a dual-targeting approach with trispecific tandem antibodies recognizing simultaneously two different TAA. As a proof of concept we have designed a *trispecific T-cell engager* (TriTE) directed against epidermal growth factor receptor (EGFR), carcinoembryonic antigen (CEA) and CD3 for colorectal cancer immunotherapy.
Materials and Methods: To generate the TriTE, an anti-EGFR and an anti-CEA single-domain VHH were cloned N-terminal and C-terminal, respectively, to an anti-CD3 scFv in the FLAG-tagged expression vector pCR3.1. Human 293T cells were transfected and conditioned medium was analyzed by Western blot, ELISA and FACS. EGFR/CD3 and CEA/CD3 bispecific light T-cell engagers (LiTE) were also generated to compare the functional activity of the three constructs.
Results: Western blot analysis demonstrated that LiTE and TriTE were secreted and the migration patterns were consistent with the predicted molecular weight (43 and 57 kDa, respectively). As shown by ELISA, the TriTE was able to bind simultaneously plastic-immobilized CEA and EGFR. Moreover, it efficiently recognized the cognate antigens of the three parental antibodies on the cell surface as assessed by flow cytometry.
Conclusions: To our knowledge, the TriTE is the first trispecific antibody simultaneously targeting two TAA and CD3 to redirect specifically T cell responses against colorectal cancer cells. This strategy opens the door for a new class of promising immunomodulatory antibodies in oncology.

P.B1.06.18
Targeted tumour treatment using polymer drug delivery systems

***M. Sirova**[1], B. Rihova[1], P. Chytil[2], M. Tavares[2], O. Lidicky[3], T. Etrych[3];*
[1]Inst. of Microbiology ASCR, Prague 4, Czech Republic, [2]Inst. of Macromolecular Chemistry ASCR, Prague 6, Czech Republic, [3]Inst. of Macromolecular Chemistry ASCR, Prague 4, Czech Republic.

Polymer-bound cytotoxic drugs represent a potential strategy of an effective tumour-targeted therapy devoid of serious side effects. Biocompatible polymer carrier ensures prolonged circulation of the drug in inactive form, its tumor-specific accumulation and controlled release in the target tissue. Water-soluble *N*-(2-hydroxypropyl)methacrylamide (HPMA) is one of the most promising drug carriers. Treatment of murine syngeneic tumours with HPMA-based conjugate of doxorubicin resulted in stronger infiltration of the treated tumours with immune cells , as compared with free doxorubicin. The infiltrates contained more CD8$^+$ effector cells than the infiltrate of Dox-treated tumours. The T cells expressed elevated levels of PD-1, indicating a possible advantage of using checkpoint inhibition. These data conform with our previous results documenting that, upon treatment with the polymer cytotoxic drugs but not with free drugs, a complete regression of the tumours can be achieved, associated with long-lasting tumour resistance, chiefly mediated by CD8$^+$ T cells. Indeed, the host immune system is co-responsible for the curative effect of the HPMA-based cytotoxic drugs. The same drug delivery system also proved good performance when delivering agents, which could reduce suppressing activity of myeloid-derived suppressor cells (MDSC), thereby potentiating the effect of polymer-bound cytotoxic drugs. Supported by projects 17-08084S and 16-28600A.

P.B1.06.19
Chondroitin sulfate proteoglycan 4 (CSPG4) as an immune target in induced drug tolerant melanoma cells (IDTCs)

***K. Uranowska**[1,2], T. Kalic[1,2], K. Karapandža[1], I. Ellinger[2], H. Breiteneder[2], H. Schaider[3], C. Hafner[1];*
[1]Department of Dermatology, Karl Landsteiner University of Health Sciences, St. Pölten, Austria, [2]Institute of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria, [3]Dermatology Research Centre, The University of Queensland Diamantina Institute, Translational Research Institute, The University of Queensland, Brisbane, Australia.

Acquired resistance to PLX4032, a selective inhibitor of mutant BRAF, remains the major obstacle in treating patients with metastatic melanoma. Induced drug-tolerant cells (IDTCs), characterized by an increase in CD271 expression, constitute a state preceding permanent drug-resistance. Chondroitin sulfate proteoglycan 4 (CSPG4) is a multifunctional transmembrane proteoglycan, involved in spreading, migration and invasion of melanoma. We hypothesize that targeting CSPG4 on IDTCs with anti-CSPG4-antibodies may delay or prevent the development of acquired drug-resistance by simultaneous interference of multiple signaling pathways.
BRAF-mutant CSPG4-positive and negative melanoma cells were exposed to PLX4032 in order to generate IDTCs. CD271 expression was monitored as a marker of IDTCs by flow cytometry. Morphological changes were analyzed by bright-field microscopy. CSPG4 expression on melanoma cells before, during and after exposure to PLX4032 was evaluated by FACS and immunofluorescence microscopy.
Exposing BRAF-mutant cells to PLX4032 led to IDTCs, which were characterized by elevated CD271 expression compared to non-treated cells and by morphological changes. A lower mean fluorescence intensity of the CSPG4 signal was found in IDTCs (1594.6±28.7) compared to untreated cells (2829.5±73.9). IF microscopy confirmed a decreased amount of CSPG4 on the IDTCs cell surfaces.
These results might indicate that the inhibition of mutant BRAF influences the expression of CSPG4. This provides the basis for further investigation of the use of CSPG4 as a potential immune target in IDTCs and gives the rationale for studying the role of CSPG4 in the development of permanent drug-resistance in melanoma cells.
Supported by: NFB(LSC15-007) and PARSS2016_NearMiss.

P.B1.06.20
Induction of cytotoxic T cell antitumor activity by combined chemo-immunotherapy with methotrexate nanoconjugates and dendritic cell-based vaccines in mice bearing MC38 colon carcinoma

***K. Węgierek**, A. Szczygieł, N. Anger, J. Mierzejewska, J. Rossowska, T. Goszczyński, M. Świtalska, E. Pajtasz-Piasecka;*
Ludwik Hirszfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wrocław, Poland.

Conjugation of methotrexate and hydroxyethyl starch (MTX-HES) is an innovative solution for prolongation of MTX half-life, reduction of its side effects and enhancing of its antitumor activity. In order to improve MTX-HES therapeutic effect, we performed *in vivo* experiments in which the chemotherapy was supplemented with bone marrow-derived dendritic cell (BM-DCs). Initially, mice with subcutaneously growing MC38 tumors received intravenously one dose of MTX or MTX-HES. On the 3rd day of chemotherapy, BM-DCs were added (in peritumoral injections, in three consecutive weeks). Mice were sacrificed three days after chemotherapy or seven days after the last injection of BM-DC, and antitumor activity of splenocytes was evaluated in *ex vivo* analyses. Chemotherapy with MTX-HES resulted in increased percentage of CD8$^+$ T cells among splenocytes, and their tumor specific-cytotoxicity compared to control- and MTX-group. This effect was intensified when the chemotherapy was supplemented with dendritic cell-based vaccines. The treatment with MTX-HES and BM-DCs increased the percentage of CD8$^+$CD107a$^+$ cells. Moreover, gathered data indicated that therapy using a combination of MTX-HES with BM-DC-vaccines was markedly effective in generation of cytotoxic T cell antitumor activity. Taken together, novel MTX-HES nanoconjugate is able to modulate the antitumor response more effectively than MTX. Meanwhile, its combination with cellular vaccines can improve both therapeutic effects and specific cytotoxic activity of splenocytes. The study was funded by National Science Centre, Poland (project no. 2015/19/N/NZ6/02908).

P.B1.07 Tumor vaccination principles and Immunotherapy - Part 7

P.B1.07.01
Targeting gangliosides-containing liposomes to human CD169+ antigen presenting cells to induce anti-tumor immune responses

***A. J. Affandi**[1], J. Grabowska[1], M. Lopez Venegas[1], K. Olesek[1], A. Barbaria[1], P. Mulder[1], M. Ambrosini[1], J. Stöckl[2], G. Storm[3], Y. van Kooyk[1], J. M. den Haan[1];*
[1]VUmc, Amsterdam, Netherlands, [2]Institute of Immunology, Vienna, Austria, [3]Utrecht University, Utrecht, Netherlands.

Pancreatic cancer forms a major cause of cancer related deaths with a very short mean overall survival of just 6-12 months. Our previous work has already demonstrated that CD169+ macrophages can stimulate superior immune responses. Using liposomes containing CD169-binding gangliosides, we hypothesized that these liposomes could be used to target and deliver antigens to human CD169+ antigen-presenting-cells (APCs). We observed that liposomes containing GM3, GD3, GM1, GD1a, GT1b, could efficiently bind and were taken up by CD169-overexpressing THP1 cells and human monocyte-derived dendritic cells (moDCs). moDC liposome binding and uptake could be further enhanced by IFN-α-induced CD169 upregulation, and blocked using neutralizing α-CD169 antibody.

Furthermore, ganglioside-containing liposome were taken up by human peripheral blood CD169+ monocytes, CD169+CD123+ and CD169+CD11c+ DCs, and splenic macrophages. The levels of uptake were associated with CD169 expression. Our ongoing experiments will evaluate the uptake and the intracellular trafficking of ganglioside-containing liposomes using imaging flow cytometry. To conclude, several ganglioside-containing liposomes bind to human CD169 and could potentially be used to target CD169+ APCs. Future studies will focus on whether these liposomes can be used to induce pancreatic tumor antigen-specific T cell responses.

P.B1.07.02

TMV vaccine inhibits growth of squamous cell carcinoma of head and neck in mice

R. Bommireddy[1], *L. E. Munoz*[1], *C. D. Pack*[2], *S. Ramachandiran*[2], *S. J. Reddy*[2], *J. Kim*[1], *G. Chen*[3], *N. F. Saba*[3], *D. M. Shin*[3], *P. Selvaraj*[1];
[1]*Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, United States,* [2]*Metaclipse Therapeutics Corporation, Atlanta, United States,*
[3]*Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, United States.*

Introduction: Head and neck cancer is a leading cause of cancer related deaths accounting for approximately 3% of all cancer related mortalities in the US. Currently, there is no cure for the advanced squamous cell carcinoma of the head and neck (SCCHN) thus development of efficacious therapies is urgently needed. To test whether vaccine-induced immunity inhibits tumor growth, we investigated efficacy of a tumor membrane-based vaccine immunotherapy in a murine SCC (SCC VII) model.
Materials and Methods: The SCC VII tumors grown subcutaneously in C3H/HeJ mice were harvested to generate tumor membrane vesicles (TMVs). TMVs were then protein transferred with glycolipid-anchored immunostimulatory molecules GPI-B7.1 and GPI-IL-12 to generate the TMV vaccine. Mice were vaccinated with TMV vaccine either before (prophylactic) or after SCC VII tumor cell challenge (therapeutic) and tumor growth was monitored every 3 days. Survival was then assessed using a Kaplan-Meier survival curve and significance determined using a Log-rank test for comparison analysis.
Results: SCC VII cells express MHC I, CD44, CD47 and respond to IFNγ in vitro. The TMV vaccine inhibited tumor growth and improved the survival of mice challenged with SCC VII tumor cells. Further, TMV vaccination induced Th1 cytokine response in vivo.
Conclusions: These observations suggest that tumor tissue-based vaccines can be harnessed to develop an effective immunotherapy for squamous cell carcinoma of the head and neck.
Funding: Supported by Head and Neck SPORE pilot funding from Emory Winship Cancer Institute.

P.B1.07.03

DNA prime-peptide boost immunizations maximize circulating and resident memory CD8⁺ T cells responses against a melanoma-associated self-antigen.

P. Caceres-Morgado*, F. Galvez-Cancino, X. Diaz, E. Menares, S. Hidalgo, O. Chovar, J. Saavedra, A. Lladser;*
Fundación Ciencia & Vida, Santiago (Ñuñoa), Chile.

Memory CD8⁺ T cell responses can mediate long-lasting protection against cancers. Emerging evidence indicates that effective anti-tumor protection requires coordinated action of both tissue-resident and circulating memory CD8⁺ T cells. However, clinically applicable vaccination strategies that efficiently establish both types of responses against tumor-associated self-antigens remain largely unexplored and are expected to strongly protect against tumors. Here we demonstrate that intradermal vaccination using a DNA prime-peptide boost strategy induces strong CD8⁺ T cell effector responses against a model of melanoma-associated self-antigen GP100 in mice, as compared to DNA and peptide prime and the other immunization regimens. These responses were characterized by a high proportion of memory precursor (KLRG1- CD127+) cells expressing CXCR3. At the memory phase, DNA prime-peptide boost lead to enhanced circulating and skin resident memory (Trm) CD8⁺ T cells. Trm cells accumulated at both vaccinated and distant sites, and displayed elevated expression of PD-1 and low expression of Lag3 and KLRG1. Interestingly, *in vivo* stimulation with H-2 Db-restricted $GP100_{(25\text{-}33)}$ peptide leads to the production of high levels of IFN-γ by Trm cells. Current efforts of this project seek to demonstrate that prime and boost vaccination strategies designed to maximize resident and circulating memory CD8⁺ T cell responses achieve potent protection against primary and disseminated melanoma.

P.B1.07.04

CD169⁺ macrophages and the development of anti-cancer vaccines

D. van Dinther[1], *J. Grabowska*[1], *A. J. Affandi*[1], *M. Lopez Venegas*[1], *A. Barbaria*[1], *H. Veninga*[1], *E. Borg*[1], *L. Hoogterp*[1], *K. Olesek*[1], *H. Kalay*[1], *M. Ambrosini*[1], *G. Storm*[2], *Y. van Kooyk*[1], ***J. M. M. den Haan***[1];
[1]*VU University Medical Center, Amsterdam, Netherlands,* [2]*Utrecht University, Utrecht, Netherlands.*

CD169-expressing macrophages are present in lymphoid organs at the site of antigen entrance and are essential in the activation of innate as well as adaptive immune responses. Our aim is to target tumor antigens to these CD169+ macrophages for the activation of anti-cancer immune responses and have investigated two approaches. First, using antiCD169 antibody-antigen conjugates we have shown that CD169+ macrophages present antigen to B cells and stimulate strong germinal center B cell and antibody responses. Furthermore, antigens targeted to CD169+ macrophages were transferred to cross-presenting dendritic cells and this led to strong cytotoxic and helper T cell responses. Both (melanoma) peptide as well as protein antigen targeting to CD169 resulted in strong primary and recall immune responses and protective immunity against melanoma outgrowth in mice. Second, we used liposomes containing CD169-binding ganglioside ligands to target antigens to CD169+ antigen presenting cells in mice and man. These liposomes specifically bound to CD169+ macrophages in mice and led to B and T cell responses against antigen present in the liposome. In addition, CD169 ligand-containing liposomes bound to human monocyte-derived dendritic cells and peripheral blood CD169+ dendritic cells and will be further evaluated for their capacity to stimulate T cell responses. In conclusion, different approaches to target tumor antigens to CD169+ antigen presenting cells demonstrate a strong capacity to stimulate immune responses and should be further explored as a vaccination strategy for cancer.

P.B1.07.05

Tumor derived microvesicles enhance crossprocessing ability of clinical grade dendritic cells

M. Dionisi*, C. De Archangelis, F. Battisti, H. Rahimi Koshkaki, I. G. Zizzari, C. Napoletano, C. Albano, I. Ruscito, M. Nuti, A. Rughetti;*
"Sapienza" University of Rome, Department of Experimental Medicine, Rome, Italy.

Introduction: Manufacturing clinical grade Dendritic Cells (DCs) represents a critical step in antitumor vaccination strategies. Indeed, DC performance is affected by clinical grade culture conditions. Thus, the design of immunogens enhancing antigen presentation of Clinical Grade DCs (cgDCs) is mandatory. Tumor-derived microvesicles (T-MVs) trigger protective anti-tumor immune responses by delivering tumor antigen repertoire and activatory signals to DCs. T-MVs modulate DC phagosomal alkalinization allowing cross-presentation of tumor glycosylated antigens such as MUC1. Here we show that phagosomal performance of cgDCs is altered (low pH, reduced phagocytosis) and that T-MVs uptake counteracts phagosomal acidification of cgDCs, restoring MUC1 antigen cross-processing.
Methods: cgDCs generated in X-Vivo medium and standard DCs (sDCs) grown in RPMI+10%FCS were pulsed with soluble recombinant MUC1 glycoprotein (rMUC1) or T-MVs carrying MUC1. Phagosomal pH and phagocytosis were assessed by flow cytometry, "chasing" DCs with 3µm FITC/FluoProbes647 coupled microbeads. Internalization and cross-processing of the MUC1 carried by T-MVs or rMUC1 were evaluated by immunofluorescence, employing HLA I and II compartment markers.
Results: cgDCs, compared to sDCs, displayed a more acidic phagosomal pH and a decreased phagocytosis. cgDCs also were less efficient in the internalization of soluble rMUC1 glycoprotein and internalized antigen was blocked in HLA class II compartment. Pulsing cgDCs with T-MVs, an increase in antigen up-take and MUC1 translocation in HLA class I compartment were observed. Indeed, T-MVs up-take triggers an early phagosomal alkalinization in cgDCs allowing MUC1 cross-processing.
Conclusions: T-MVs are able to reprogram DC antigen presenting machinery and represent optimal cell-free based immunogens for clinical use.

P.B1.07.06

Generation of multiepitope cancer vaccines based on large combinatorial libraries of survivin-derived mutant epitopes

A. N. Domínguez-Romero*, M. E. Munguia-Zamudio, F. Martínez-Cortés, K. Manoutcharian;*
Instituto de Investigaciones Biomédicas, México CDMX, Mexico.

Introduction: Immune tolerance is the main challenge in the field of cancer vaccines, therefore, mutated versions of a wild-type epitope of tumor-associated antigens represent a promising pathway for these vaccines. A novel vaccine approach has been developed by our research group based on the generation of a new class of vaccine immunogens, called Variable Epitope Libraries (VELs) bearing combinatorial libraries of mutated versions of wild-type immunodominant epitopes of cytotoxic T lymphocytes. Previously, we demonstrated statistically significant tumor growth inhibition in BALB/c mice immunized with the VELs based on a $survivin_{66\text{-}74}$ immunodominant epitope. Now, we used larger regions of survivin (40-45 amino acid long) to generate the VELs as multiepitope vaccines.
Materials and Methods: These VELs were expressed at high copy numbers on recombinant M13 bacteriophages as peptides linked to the cpVIII phage protein by Phage Display. A 4T1 murine breast cancer model was used. The VEL vaccines were applied in therapeutic treatment studies and administered as a single intravenous injection.Results: Our preliminary data showed that a single dose of the survivin-based VEL vaccine significantly decreased tumor growth and lung metastasis. Also, a significant reduction of MDSC cells in the lungs and tumor infiltration by CD8 T lymphocytes were observed. Conclusions: This study provides the feasibility of the generation of VEL-based vaccine immunogens as an alternative approach for the construction of cancer epitope vaccines.
Acknowledgments: Funding provided by DGAPA-UNAM (IN205216). A. Domínguez-Romero is a recipient of a scholarship from CONACyT and Posgrado en Ciencias Biológicas, UNAM.

P.B1.07.07

Using combinatorial peptide libraries to design super-agonists for improved cancer immunotherapy

S. A. E. Galloway[1], *G. Dolton*[1], *B. Szomolay*[1], *A. Wall*[1], *V. Bianchi*[1], *M. Attaf*[1], *P. J. Rizkallah*[1], *M. Donia*[2], *I. M. Svane*[2], *P. Thor Straten*[2], *C. Rius*[1], *A. Fuller*[1], *A. K. Sewell*[1];
[1]*Cardiff University, Cardiff, United Kingdom,* [2]*Centre for Cancer Immune Therapy, Copenhagen, Denmark.*

The use of peptide vaccines for the treatment of melanoma has been a largely unsuccessful endeavour. Generally, peptides derived from tumour associated antigens (TAA) are poorly immunogenic and unable to elicit a substantial CD8 T-cell response capable of mediating effective tumour regression. Here, we demonstrate that combinatorial peptide library (CPL) screens are an important tool to design agonist peptides capable of improving the CD8 T-cell response to a commonly overexpressed TAA, melan-A.
Combinatorial peptide library (CPL) screening of key CD8 T-cell clones, isolated from a patient who successfully underwent tumour infiltrating lymphocyte (TIL) therapy, revealed preferred amino acid residues at each position in the cognate peptide, leading to the selection of a panel of 10 potential agonist peptides.
Testing of candidate peptides using polyclonal T-cell populations and a panel of melan-A specific T-cell clones highlighted 2 dominant agonist peptides. The chosen peptides elicited superior expansion of wild type pMHC-tetramer-positive CD8 T-cells from polyclonal T-cell populations compared to the wild-type peptide in ten HLA A2+ donors tested. ^{51}Cr release assays demonstrated that CD8 T-cells primed with agonist peptides could effectively kill melanoma tumour cells.
Polymorphonuclear cells (PBMC) from patients with melanoma will now be used to assess whether agonist peptides can improve CD8 T-cell responses to autologous tumour. This study highlights the use of CPL screening technology to design superior peptides capable of enhancing the CD8 T-cell response to melanoma.

P.B1.07.08

Anti angiogenic Effects of CD73 specific siRNA Loaded Nanoparticles in Breast Cancer Bearing Mice

G. Ghalamfarsa[1], *A. Rastegari*[2], *F. Atyabi*[2], *H. Hassannia*[3], *M. Hojjat-Farsangi*[4,5], *A. Ghanbari*[1], *J. Mohammadi*[6], *A. Masjedi*[7,8], *M. Yousefi*[8], *B. Yousefi*[9], *J. Hadjati*[9], *F. Jadidi-Niaragh*[10,8,9];
[1]*Yasuj University of Medical Sciences, Cellular and Molecular Research Center, Yasuj, Iran, Islamic Republic of,* [2]*Nanotechnology Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran, Tehran, Iran, Islamic Republic of,* [3]*Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran, Tehran, Iran, Islamic Republic of,* [4]*Department of Oncology-Pathology, Immune and Gene therapy Lab, Cancer Center Karolinska (CCK), Karolinska University Hospital Solna and Karolinska Institute, Stockholm, Sweden, Stockholm, Sweden,* [5]*Department of Immunology, School of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran, Bushehr, Iran, Islamic Republic of,* [6]*Medicinal Plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran, Yasuj, Iran, Islamic Republic of,* [7]*Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, Tabriz, Iran, Islamic Republic of,* [8]*Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran, Tabriz, Iran, Islamic Republic of,* [9]*Department of Immunology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran, Tehran, Iran, Islamic Republic of,* [10]*Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran., Tabriz, Iran, Islamic Republic of.*

Increased expression of adenosine in the tumor microenvironment is markedly related to the overexpression of its molecular producer, CD73. It has been demonstrated that overexpression of CD73 facilitates the tumor growth, angiogenesis, and metastasis processes. Regarding the importance of angiogenesis in tumor development and spreading, we decided to assign the anti-angiogenic effects of CD73 suppression, both in vitro and in vivo. We used chitosan lactate (ChLa) nanoparticles (NPs) to deliver CD73 specific siRNA into cancer cells. Our results showed that treatment of 4T1 cells with CD73-specific siRNA loaded NPs led to potent inhibition of cell proliferation and cell cycle arrest which was associated with partial apoptosis induction, in vitro. This growth arrest was correlated with downregulation of angiogenesis-related molecules including VEGF-A, VEGF-R2, IL-6, and TGF-β. In addition, silencing CD73 could effectively suppress the migration and invasion capacity of tumor cells. Moreover, administration of NPs loaded with CD73-siRNA into 4T1 breast cancer bearing mice led to arrested tumor growth and increased mice survival time which was associated with downregulation of the mRNA expression levels of angiogenesis (VEGF-A, VEGF-R2, VE-Cadherin, and CD31) and lymphangiogenesis (VEGF-C and LYVE-1) related factors in the tumor tissues. Furthermore, the expression of angiogenesis promoting factors IL-6, TGF-β, STAT3, HIF-1α, and COX2 was decreased after CD73 suppression in mice. These results indicate that suppression of tumor development by downregulation of CD73 is in part related to angiogenesis arrest. These findings imply a promising strategy for inhibition tumor growth accompanied by suppression of angiogenesis process.

P.B1.07.09

Evaluation of GM3-containing liposomes for antigen targeting to splenic CD169+ macrophages to induce anti-cancer immunity

J. Grabowska[1], *D. van Dinther*[1], *K. Olesek*[1], *L. Hoogterp*[1], *M. Ambrosini*[1], *P. R. Crocker*[2], *G. Storm*[3], *Y. van Kooyk*[1], *J. M. den Haan*[1];
[1]*VUmc, Amsterdam, Netherlands,* [2]*University of Dundee, Dundee, United Kingdom,* [3]*Utrecht University, Utrecht, Netherlands.*

Liposomes are an attractive antigen delivery system and have been successfully used for vaccination strategies. We previously have shown that antibody-mediated antigen (Ag) targeting to CD169+ macrophages stimulates superior Ag-specific CD8 T cell responses. CD169, also known as sialoadhesin or siglec-1, is a sialic-acid binding lectin which has been described to bind to ganglioside GM3. Here we tested GM3-containing liposomes for their binding and uptake by murine CD169+ macrophages and their capacity to induce immune responses. As expected, CD169+ macrophages strongly and specifically bound GM3 liposomes, while macrophages from sialoadhesin knock-in mice bearing a mutation in the CD169 ligand binding pocket were incapable of binding GM3 liposomes. After intravenous administration, GM3/ovalbumin-containing liposomes specifically bound to CD169+ macrophages in a sialic acid dependent manner and stimulated ovalbumin-specific CD8 and CD4 T cell and B cell responses. Surprisingly, liposomes without GM3 also stimulated immune responses, while not binding to CD169+ macrophages. In our current studies we will also evaluate liposomes that contain the ganglioside GM1, that does not bind CD169, for their capacity to evoke an immune response. In future studies we will determine the efficacy of GM3 liposomes to target tumor antigens to CD169+ macrophages in order to induce anti-cancer immune responses.

P.B1.07.10

Photochemical internalization as a novel strategy to realize therapeutic cancer peptide vaccination

M. Haug[1,2,3], *G. Brede*[2], *M. Håkerud*[4], *A. Nedberg*[4], *O. A. Gederaas*[2], *V. T. Edwards*[4,5], *T. H. Flo*[1,2,6], *P. K. Selbo*[4], *A. Høgset*[5], *Ø. Halaas*[2];
[1]*Centre of Molecular Inflammation Research, NTNU, Trondheim, Norway,* [2]*Department of Clinical and Molecular Medicine, NTNU, Trondheim, Norway,* [3]*Department of Infection, St. Olavs University Hospital, Trondheim, Norway,* [4]*Department of Radiation Biology, Oslo University Hospital, Oslo, Norway,* [5]*PCI Biotech AS, Oslo, Norway,* [6]*5Centre for Molecular Medicine Norway, Nordic EMBL Partnership, University of Oslo, Oslo, Norway.*

Effective priming and activation of tumor-specific CD8+ cytotoxic T lymphocytes (CTLs) is crucial for realizing the potential of therapeutic cancer vaccination. This requires cytosolic antigens that feed into the MHC class I presentation pathway, which is not efficiently achieved with most current vaccination technologies. Photochemical internalization (PCI) provides an emerging technology to route endocytosed material to the cytosol of cells, based on light-induced disruption of endosomal membranes using a photosensitizing compound. Here we investigated the potential of PCI as a novel, minimally invasive and well-tolerated vaccination technology to induce priming of cancer-specific CTL responses to peptide antigens.
We show that PCI effectively promotes delivery of peptide antigens to the cytosol of antigen presenting cells *in vitro*. This resulted in a 30-fold increase in MHC class I/peptide complex formation and surface presentation, and a subsequent 30-100-fold more efficient activation of antigen-specific CTLs compared to using the peptide alone. The effect was found to be highly dependent on the dose of the PCI treatment, where optimal doses promoted maturation of immature dendritic cells, thus also providing an adjuvant effect.
The effect of PCI was confirmed *in vivo* by the successful induction of antigen-specific CTL responses to cancer antigens in C57BL/6 mice following intradermal peptide vaccination using PCI technology. We thus show new and strong evidence that PCI technology holds great potential as a novel strategy for improving the outcome of peptide vaccines aimed at triggering cancer-specific CD8+ CTL responses.

P.B1.07.11

Discovery of immunogenic neoantigens for peptide vaccination approaches in murine colorectal cancer.

B. J. Hos;
IHB, LUMC, Leiden, Netherlands.

Recent developments have shown that effectiveness of therapy with checkpoint-blocking antibodies correlates with the expansion and invigoration of neo-antigen specific T cells. Alongside, peptide-based vaccines targeting onco-viral antigens have shown to be effective inducers of T cell responses related to reduction of HPV-induced pre-malignancies. This suggests that peptide-based vaccination targeting neoantigens is a viable immunotherapeutic strategy.
A major limitation for broad application of peptide-based vaccinations is the characterization of cancer-specific epitopes that is required for personalized approaches. The process of epitope identification is yet not trivial. Here we describe the process in a murine colorectal cancer model to identify immunogenic epitopes for peptide vaccination.
DNA and RNA analysis revealed the expression of several thousand mutations. Prediction and ranking of MHC class I binding peptides containing mutations with NetMHC4.0 limited this number to several hundred high- and moderate-affinity peptides. An immunogenicity test of 57 selected high-affinity peptides (based on the type of amino-acid substitution) revealed a large percentage of peptides induced CD8- and CD4-specific responses. However, ex vivo restimulation and subsequent readout of splenocytes from anti-PD-L1 treated and tumor-regressed mice, showed specific responses to a limited number of three peptides. Strong responses were observed to a novel mutated peptide sequence. Mass spectrometry could confirm the expression and presentation of this epitope, but not the other two.
Our approach was successful in the characterization of immunogenic and relevant epitopes. Further research is now needed to improve effectivity in therapeutic vaccinations.

P.B1.07.12

Identifying the role of microRNAs in MDS with particular focus on the contribution of immune cells

A. Kindermann[1], *F. Heidel*[2], *H. Kielstein*[1], *D. Quandt*[1];
[1]*Department of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg, Germany,* [2]*Department of Hematology and Medical Oncology, Friedrich-Schiller University Jena, Germany.*

Introduction: Myelodysplastic syndromes (MDS) are clonal hematopoietic neoplasia characterized by peripheral cytopenia with defective hematopoiesis and variable tendency to evolve into acute leukemia. MicroRNAs are known to be tight regulators of normal hematopoiesis but also to be dysregulated in various types of cancer and therefore are targets of current therapeutic approaches. MiR-34a is differentially expressed in MDS but the molecular mechanism remains elusive. Altered microRNA expression can evolve from and affect all different cell types that constitute the stem cell niche. **Methods:** Allogeneic tumor-T cell co-culture assays with myeloid leukemic as well as MDS cell lines could be successfully established. By the use of Viromer transfection we were able to overexpress miR-34a using mimics in primary human T cells and leukemic cell lines as determined by qPCR. Differential bone marrow cell sorting for early hematopoietic stem cells (CD34+, lin neg, CD38 neg), committed stem cells as well as T cells will allow the generation of distinct microRNA profiles. **Results:** Overexpression of miR-34a did not alter subsequent proliferative behaviour of polyclonal activated T cells. Tumor-T cell co-culture assays with altered miR-34a expression on the tumor site did not yet give conclusive results of an impact on T cell activation. The FACS sorting strategy for differential bone marrow cells could be established and high quality RNA from 100 cells could be isolated. **Conclusion:** MiR-34a seems not to contribute primarily to T cell-tumor communication. The role of this microRNA for myeloid progenitor survival, proliferation and differentiation will be investigated in ongoing experiments.

P.B1.07.13

Dendritic cells pulsed with tumor cells killed by high hydrostatic pressure are effective as prostate cancer immunotherapy using clinically relevant murine models

R. Mikyskova[1], *I. Stěpanek*[1], *I. Truxova*[2], *I. Moserová*[2], *J. Fučikova*[3,2], *I. Kanchev*[1], *J. Bartůňková*[3,2], *R. Spisek*[3], *M. Reinis*[1];
[1]*Institute of Molecular Genetics AS CR, v.v.i., Prague 4, Czech Republic,* [2]*SOTIO, Prague 7, Czech Republic,* [3]*2nd Faculty of Medicine and University Hospital Motol, Charles University, Prague 5, Czech Republic.*

High hydrostatic pressure (HHP) has been proved to induce immunogenic cell death of cancer cells and dendritic cell (DC)-based vaccines pulsed with HHP-inactivated tumor cells have recently been shown to be a promising tool for immunotherapy. In this study, we analyzed their therapeutic efficacy in clinically relevant settings, such as chemoimmunotherapy, surgical minimal residual tumour disease and, finally, in the orthotopic transgenic adenocarcinoma of the mouse prostate (TRAMP) model, which mimics well humans carcinoma as it develops and progresses through all stages of carcinogenesis similarly as in humans. DC-based vaccines pulsed with the HHP-inactivated tumour cells combined with docetaxel (DTX) chemotherapy inhibited growth of both TRAMP-C2 and TC-1 tumors. Administration of these vaccines after the surgical removal of tumors slowed down the growth of tumour recurrences. Finally, pulsed DC-based vaccines were also effective in reducing prostate cancer growth in the TRAMP model when used alone or in the combination with docetaxel. Although we did not observe any additive or synergic effects of chemoimmunotherapy on the tumor growth, the combination of DTX and pulsed dendritic cells resulted in significantly lower tumour cells proliferation (detected by Ki67 staining) in growing tumors. Collectively, our results indicate that the DC-based vaccines pulsed with HHP-inactivated tumor cells represent a suitable tool for immunotherapy, particularly with regard to the findings that poorly immunogenic TRAMP-C2 tumors were susceptible to this treatment modality. This work was supported by research grants provided by SOTIO a.s. and by Academy of Sciences of the Czech Republic (RVO 68378050).

P.B1.07.14

The *in vitro* melanoma tumor microenvironment conditions macrophages to an immunosuppressive M2-like phenotype, which is reversible by lipopolysaccharide and interferon-gamma

I. Milenova[1], *M. Lopez Gonzalez*[2], *T. Brachtlova*[2], *R. van de Ven*[1], *W. Dong*[3], *V. W. van Beusechem*[2], *T. de Gruijl*[2];
[1]*Vrije Universiteit Medical Center/ORCA Therapeutics, Amsterdam, Netherlands,* [2]*Vrije Universiteit Medical Center, Amsterdam, Netherlands,* [3]*ORCA Therapeutics, Amsterdam, Netherlands.*

The melanoma tumor microenvironment is conditioned to skew infiltrating monocytes to M2-like macrophages. These M2-like macrophages promote growth and invasion of tumor cells. We are exploring the use of oncolytic adenoviruses to enhance the efficacy of immunotherapy and induce favorable M1-like polarization.
In order to study macrophage polarization in the tumor microenvironment, human melanoma cell lines were co-cultured *in vitro* with $CD14^+$ monocytes. Lipopolysaccharide (LPS) and interferon (IFN)-gamma were added to the co-culture to assess M1 polarization, and the effect of the oncolytic adenovirus ORCA-010, which is modified to facilitate improved spread in tumor cells, was studied.
We show that the melanoma cells strongly induce an M2-like macrophage phenotype (CD14+/CD163+/CD80-/CD86-/PD-L1+) *in vitro*. Whereas addition of ORCA-010 led to upregulation of CD163, the combination of LPS and IFN-gamma proved able to negate these effects and induce an M1-like phenotype (CD14-/CD163-/CD80+/CD86+/PD-L1++). These findings demonstrate the feasibility of M1 skewing in the face of melanoma-induced immune suppression.
Next, the macrophage polarizing effects of various clinically applicable immune modulatory agents will be studied. In the B16-OVA melanoma model we are investigating the effects of the combined administration of ORCA-010, a p38-MAPK inhibitor and anti-PD-1. We have observed *in vitro* inhibition of p38 signaling to skew monocyte differentiation away from a melanoma-imposed immunosuppressive M2-like phenotype. We hypothesize that this will result in increased T cell infiltration and activation at the tumor site, thus increasing the efficacy of PD-1 checkpoint blockade.
This work is supported by the European Union Horizon2020 ITN-EID grant 643130 “VIRION”.

P.B1.07.15

***Are you my MAIT?*- Investigating the role of MAIT cells in Colorectal-Liver Metastases**

R. M. Millen[1], *S. Roth*[2], *P. Beavis*[2], *B. Thomson*[3], *S. Banting*[4], *B. Knowles*[1], *J. Kelly*[5], *N. A. Gherardin*[6], *D. Godfrey*[6], *K. Visvanathan*[4], *R. G. Ramsay*[2];
[1]*Peter MacCallum Cancer Centre/St. Vincent’s Hospital, Melbourne, VIC, Australia,* [2]*Peter MacCallum Cancer Centre, Melbourne, VIC, Australia,* [3]*Peter MacCallum Cancer Centre/ Royal Melbourne Hospital, Melbourne, VIC, Australia,* [4]*St. Vincent’s Hospital, Melbourne, VIC, Australia,* [5]*The Fiona Elsey Cancer Research Institute, Ballarat, VIC, Australia,* [6]*Peter Doherty Institute, Melbourne, VIC, Australia.*

Background: Stage-IV Colorectal Cancer (CRC) involves metastasis to the liver. Cytotoxic CD8+ T-cells play a critical role in cancer; serving as a key prognostic marker for the Galon-Immunoscore™
Mucosal-Associated Invariant T-cells (MAITs) are a recently described subset of T-cells that co-express CD8, and are important in controlling bacterial infections. MAITs are highly abundant in the liver comprising up to 40% of T-cells. The role of MAITs in solid tumours is starting to emerge and it’s likely they are included with CD8+ cells when defining Tumour Infiltrating Lymphocytes (TILs). MAIT cells may be prognostic and may be a novel immune-therapeutic target.
Methods: Recruit 40 patients with colorectal-liver metastasis and investigate the role of MAIT cells in the tumour, normal liver and peripheral blood by flow cytometry. Assess function using tumour cell killing assays, developed in our lab, with patient-derived tumouroid cultures.
Results: We have recruited 25 patients with liver mets and documented MAITs in the tumour; surrounding liver tissue and PBMCs. MAIT cell frequency is decreased in the tumour compared to the surrounding tissue. However, MAITs have a high expression of PD-1 in the tumour, indicating potential response to immune checkpoint blockade (ICB). We have preliminary data demonstrating that autologous MAIT cells have effector function when co-cultured with patient-derived tumouroids *in vitro.*
Conclusions: MAITs are decreased in the tumour, with high PD-1 expression and may be ideal targets for ICB. These intriguing results warrant further investigation to determine their direct or indirect role of MAIT cells in tumour immunity.

P.B1.07.16

Virus like particle vaccine targeting xCT protein in preclinical breast cancer models

V. Rolih[1], *E. Bolli*[1], *L. Conti*[1], *J. M. Christen*[2], *J. Caldeira*[2], *F. Pericle*[2], *F. Cavallo*[1];
[1]*University of Turin, Turin, Italy,* [2]*Agilvax Inc., Albuquerque, United States.*

Cancer stem cells (CSCs) are involved in the resistance mechanism to traditional radio- and chemo-therapies. They represent a reservoir for relapse and metastatic evolution.
The cystine-glutamate antiporter xCT (SLC7A11) has been identified as over-expressed on the cell surface of breast CSC (BCSC) and essential for their resistance to common cytotoxic therapies. xCT expression is restricted to a few normal cell types, in particular neurons and subset of macrophages. In breast cancer patients, high levels of xCT mRNA and protein correlate with significant reduction in distal metastases-free and overall survival. Thus, xCT could be an ideal oncoantigen for immunotherapy. We have developed an innovative vaccine based on a virus-like-particle (VLP) technology targeting the xCT protein for breast cancer treatment.
Immunization with our VLPs elicited a strong antibody response against xCT and these antibodies affected BCSC function and biology *in vitro*. We studied the effect of our vaccine on metastasis formation in two different settings: as a preventive model in BALB/c mice injected with Her2+ TUBO cells and as a therapeutic model in BALB/c mice injected with triple negative 4T1 cells. For both protocols, treated mice had a significant reduction in lung metastases compared to controls.
These data show that our VLP vaccine can inhibit xCT activity, impact CSC biology and significantly reduce metastatic progression in preclinical models. We are studying the association of our VLPs vaccine with anti-PD-1 monoclonal antibody therapy. In the future, we would like to translate this combinatorial approach to the clinical settings.

P.B1.07.17

Selection of tumor cells in the cultivation stage, suitable for the development of a vaccine against urothelial cancer

T. Slavyanskaya[1], *S. Salnikova*[2];
[1]*Peoples Friendship University of Russia (RUDN University), MOSCOW, Russian Federation,* [2]*FSBI Clinical Hospital №1 of the Presidential Affairs Management of the Russian Federation, MOSCOW, Russian Federation.*

Introduction: The development of vaccines for urothelial carcinoma (UCV) covers many aspects necessary for its standardization. Materials and Methods: We assayed UC samples from 25 patients aged 37-82. Cultivation of UC cells (UCC) was performed on growth media DMEM/F12. Evaluation of viability (V) was performed every 24 h for 6 days. Tumor disaggregation was performed by means of 2 methods: automatic mechanic and enzymatic methods. Cancer-testicular antigens (CTA) expression was evaluated at FACS Canto II device. Software package SPSS 23.0 for Windows was used. Results: V of UCC in a 1st day exposure at a temperature (T) 0^0C was fall down on 7.14±0.7%, at +4°C on 3.3±0.4% , at +8°C on 11,8±1.5% (p≤0.05). From 2 to 6 days V was decreased on 9.0±1.9% and on 66.7±4.7%, respectively. V of the UCC during the mechanical disaggregation was significantly higher than 81,8±6% (p≤0.05) compared to the enzymatic method: 28,4±3,3% or 55,6±4,9%, depending of UC forms. In UCC cultures CTA expression was detected frequently: MAGE-70%; BAGE-30%; GAGE-40%; NY-ESO-1-50%. At early passages quantity of UCC, expressing CTA, amounted to 78.0% (p<0.05). While expression of CTA at UCC more, than 30 passages, was significantly lower - 28.2±4.6% (p<0.05). Conclusions: It was worked out optimal parameters for preservation of viability of CUC in the process of cultivation, transportation and storage. UCC with high frequency of CTA expression may be used for preparation of UCV.
The publication has been prepared with the support of the "RUDN University Program 5-100".

P.B1.07.18

Genetic mutations of tumor cells depending on the clinical forms of urothelial cancer

T. Slavyanskaya[1], *S. Salnikova*[2];
[1]*Peoples' Friendship University of Russia (RUDN University), Moscow, Russian Federation,* [2]*2FSBI Clinical Hospital №1 of the Presidential Affairs Management of the Russian Federation, Moscow, Russian Federation.*

Introduction: The objective of the work is to perform comparative study of genetic mutations and expression of cancer-testicular antigens (CTA) in case of different forms of UC. Materials and Methods: The tumor specimens (TS) of UC were studied in 54 patients aged 37-82 with muscular invasive (MIF, TS-75%) and muscular non-invasive (MNIF, TS-25%) forms. Expression of CTA was assessed by FlowCytometry method. We used the package of statistical software SPSS 23.0 for Windows. Results: It was established, that all researched TS had cytogenetic changes: deletion of 9 chromosome (Ch) - 66.7%, absence of Y Ch (50%) and monosomy of 13 Ch (33.3%). In rare cases changes in 1,3,7 Ch, monosomy of 17 Ch, trisomy of 7 Ch were detected. In case of prolonged passaging in a part of cultures, significant increase of the number of genetic changes in a form of division of previously homogeneous population into subclones differing in ploidy (up to 56 Ch) and quantity of changed Ch is observed. Certain correlation of these changes to decrease of CTA expression (GAGE, BAGE, MAGE, NY-ESO-1) (p<0.05) was established. Conclusions: Thus, in MNIF UC CTA expression is significantly lower, than in case of MIF. Particular cultures in the course of multiple transits preserved cytogenetic profile and consistent CTA expression, which makes them promising for preparation of antitumor vaccine in case of UC. *The publication has been prepared with the support of the "RUDN University Program 5-100".*

P.B1.07.19

Immunisation with a synthetic vaccine consisting of a tumour-associated MUC1-glycopeptide conjugated to Tetanus Toxoid significantly reduced breast tumour growth

N. Stergiou[1], *N. Gaidzik*[2], *A. Heimes*[3], *S. Dietzen*[1], *P. Besenius*[2], *J. Jäkel*[4], *W. Brenner*[3], *M. Schmidt*[3], *H. Kunz*[2], *E. Schmitt*[1];
[1]*Institute of Immunology, Mainz, Germany,* [2]*Institute of Organic Chemistry, Mainz, Germany,* [3]*Departement of Obstetrics and Women's Health, Mainz, Germany,* [4]*Institute of Pathology, Mainz, Germany.*

Immunisation against strongly expressed tumour-associated endogenous antigens is considered to be an attractive strategy for the induction of a curative immune response concomitant with a long lasting immunological memory. The mucin MUC1 is a very promising tumour antigen, as its tumour-associated form significantly differs from the glycoprotein form expressed on healthy cells. Due to aberrant glycosylation in tumour cells, the specific peptide epitopes in its backbone are accessible and can be bound by antibodies induced by vaccination. Breast cancer patients develop *per se* only low levels of T cells and antibodies recognizing tumour-associated MUC1 and clinical trials with tumour-associated MUC1 gained unsatisfactory therapeutic effects indicating urgent need to improve humoral immunity against this tumour entity. Herein, we demonstrate that preventive vaccination against tumour-associated human MUC1 results in strong specific humoral immune responses, in a marked slowdown of tumour progression and in an increased survival of breast tumour-bearing mice. For preventive vaccination, we used a synthetic vaccine containing a specific tumour-associated glycopeptide structure of human MUC1 coupled to Tetanus Toxoid. The glycopeptide consists of a 22mer huMUC1 peptide with two immune dominant regions (PDTR and GSTA), glycosylated with the sialylated carbohydrate ST_N on serine-17. PyMT and human MUC1-double transgenic mice expressing human tumour-associated MUC1 on breast tumour tissue served as a preclinical breast cancer model.

P.B1.07.20

Tumor vaccination: chitosan nanoparticles to improve the antigen uptake by dendritic cells for an enhanced tumor-directed immune response

E. Winter[1], *O. Helm*[1], *M. Lettau*[2], *J. Heidland*[3], *R. Scherließ*[3], *F. Walter*[1], *S. Sebens*[1];
[1]*Institute of Experimental Cancer Research, Christian-Albrechts-University (CAU) Kiel and University Hospital of Schleswig-Holstein (UKSH) Campus Kiel, Kiel, Germany,* [2]*Institute of Immunology, Christian-Albrechts-University (CAU) Kiel and University Hospital of Schleswig-Holstein (UKSH) Campus Kiel, Kiel, Germany,* [3]*Department of Pharmaceutics and Biopharmaceutics, Christian-Albrechts-University (CAU) Kiel, Kiel, Germany.*

Tumor vaccination is a promising approach for treatment of tumor diseases.
Dendritic cells (DCs) play a key role in the initiation of an anti-tumor immune response. They present endocytosed tumor antigens via cross-presentation on major histocompatibility complex class I molecules thereby activating CD8+ cytotoxic T lymphocytes.
However, convincing results from studies on solid tumors are still missing. One reason might be an inefficient antigen uptake by DCs.
The aim of this study was to assess the suitability of chitosan nanoparticles (CNPs) for improving the antigen uptake by different antigen presenting cells to further enhance tumor-directed immune responses. CNPs can be used as carriers for noninvasive drug delivery, such as nasal or pulmonary administration.
Hence, the uptake of FITC-conjugated CNPs of different sizes by human DCs or macrophages was analyzed by flow cytometry revealing a high uptake rate by DCs and a less efficient uptake by macrophages. Furthermore, to confirm intracellular CNP-localization in DCs, ImageStream® analysis was performed showing a high internalization rate of nanoparticles.
In order to further investigate the suitability for inhalation, target specificity of CNPs was studied. Nanoparticle uptake analyzed in DCs, which were cocultured with human lung epithelial cells H441, showed CNP-uptake by both cell types. Current studies investigate the uptake of antigen loaded CNPs in cocultured DCs and whether this leads to potent T cell activation.
Overall, these data indicate that CNPs are efficiently internalized in DCs supporting their suitability as vehicles to improve antigen uptake by human DCs.

P.B1.08 Tumor vaccination principles and Immunotherapy - Part 8

P.B1.08.01

Natural compounds as modulators of regulatory T cell function for the treatment of cancer

F. Al-Naimi, *L. Thoele, J. Bartel, M. Guderian, M. Swallow, L. Almeida, T. Sparwasser;*
Institute of Infection Immunology, TWINCORE, Centre for Experimental and Clinical Infection Research, Hanover, Germany.

Regulatory T cells (Tregs) play a major role in maintaining the immune homeostasis of the host. However, their increased frequency observed in tumor patients and murine tumor models is associated with poor prognosis and inhibited therapy response rates. Therefore, their manipulation is still a major focus of current research. In recent projects we could show that natural compounds derived from micro-organismal secondary metabolites can influence the function of immune cells, especially T cells. In this project we identified a new additional compound that shows promising potential to inhibit regulatory T cells. First data indicated that this compound inhibits the differentiation of Tregs *in vitro* by dampening the levels of FoxP3 protein, but not FoxP3 mRNA levels. Moreover, the compound did not impair the expression of IFNγ in Th1 polarized CD4 T cells *in vitro*. Furthermore, *in vivo* experiments showed that the therapeutic application of this substance during the early stages of B16 melanoma development in mice reduced the tumor growth rate, decreased Treg frequencies in the tumor-infiltrating lymphocyte (TILs) population and increased the CD8/Treg ratio in the TILs. Besides, RNA microarray analysis of iTregs treated with the compound further supports the idea that the compound regulates FoxP3 on a posttranscriptional or even posttranslational level. Moreover, first human data in *in vitro* induced Tregs from healthy donors cultured in the presence of the compound showed a decrease of FoxP3 protein levels. Therefore, we propose that this new compound has promising immunomodulating capacity on Tregs useful for immunotherapy of melanoma.

P.B1.08.02
CD74, invariant chain, a modulator of endosomalmaturation

O. Bakke[1], *S. Walchli*[2];
[1]*Department of Biosciences, Oslo, Norway,* [2]*Sebastien Walchli, Oslo, Norway.*

0 0 1 148 849 Universitetet i Oslo 7 1 996 14.0 Normal 0 false false false NO-BOK JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin:0cm; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:Cambria; mso-ascii-font-family:Cambria; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Cambria; mso-hansi-theme-font:minor-latin;} Invariant chain (CD74, Ii) is an MHC II associated multifunctional molecule expressed in antigen presenting cells. CD74, with its two leucine based endosomal sorting signals that binds AP1 and AP2 adaptors was first found to be an essential partner for the proper trafficking of MHC II and efficient antigen loading. The trimeric CD74 delays furthermore endosomal maturation and is essential for forming the peptide loading compartment. CD74 has endosomal membrane fusion properties independent of Rab5, PI3 kinase and EEA1 and I will discuss how this property can be exploited to study maturation, endosomal fusion, fission and endosomal membrane kinetics after tyrosine kinase receptor activation. Interestingly, CD74 has been found to interact with several molecules including MHCI and can be used as a vector for simultaneously increasing both MHCI and MHCII mediated immune responses towards specific antigens and is ready to be tested in clinical therapeutic DC based cancer immunotherapy. <!--EndFragment-->

P.B1.08.03
Targeting myeloid derived suppressor cells with all-trans retinoic acid is highly time-dependent in therapeutic tumor vaccination

A. Heine[1,2], *C. Flores*[1,2], *H. Gevensleben*[3], *L. Diehl*[4,5], *M. Heikenwälder*[6,7], *M. Ringelhan*[8], *K. Janssen*[9], *U. Nitsche*[9], *N. Garbi*[2], *P. Brossart*[1], ***T. Baumann***[10], *P. A. Knolle*[10,5], *C. Kurts*[2], *B. Höchst*[10,5];
[1]*Medical Clinic III for Oncology, Hematology and Rheumatology, University Hospital, Bonn, Germany,* [2]*Institute of Experimental Immunology, University Bonn, Bonn, Germany,* [3]*Institute of Pathology, University Bonn, Bonn, Germany,* [4]*Institute of Experimental Immunology and Hepatology, University Medical Center, Hamburg-Eppendorf, Germany,* [5]*Institute of Molecular Medicine, University Bonn, Bonn, Germany,* [6]*Institute of Virology, Technische Universität München, Munich, Germany,* [7]*Division of Chronic Inflammation and Cancer, German Cancer Research Center, DKFZ, Heidelberg, Germany,* [8]*Department for Internal Medicine 2, Klinikum rechts der Isar, Technische Universität München, Munich, Germany,* [9]*Department of Surgery, Technische Universität München, Munich, Germany,* [10]*Institute of Molecular Immunology, Klinikum rechts der Isar, Munich, Germany.*

Therapeutic vaccination protocols aiming to improve adaptive immune responses present a promising anti-tumor therapy. However, T cell mediated effector functions are often counteracted by regulatory T cells (T_{regs}) and myeloid-derived suppressor cells (MDSCs).
Here, we studied therapeutic vaccination in two different mouse tumor models, B16-OVA (melanoma) and MC38-CEA (colorectal carcinoma).
Combined application of the TLR9-ligand CpG and the NKT-ligand α-GalCer induced potent anti-tumor responses in only a subpopulation of mice. Non-responders had equal levels of T_{regs}, $CD4^+$ and $CD8^+$ T cells, B cells and NK cells but elevated levels of $CD11b^+$ $MHC\text{-}II^-$ $Ly\text{-}6G^-$ $Ly\text{-}6C^{high}$ monocytic MDSCs (M-MDSCs). On a per cell basis, M-MDSCs from responders and non-responders were equal suppressive towards T cells. To overcome M-MDSC mediated suppression, concurrent and time-delayed injection of all-trans-retinoic-acid (atRA) was tested, a treatment previously reported to reduce M-MDSC numbers. Strikingly, only 3d time-delayed but not concurrent administration of atRA reduced tumor growth and improved overall survival. M-MDSCs up-regulated MHC class II, cross-presented antigens and lost their suppressive effect towards T cells. In contrast, simultaneous treatment with atRA reduced the beneficial effects of therapeutic vaccination and did not lead to MHC class II expression. Similarly, MDSCs from human colorectal carcinoma patients failed to up-regulate HLA-DR after TLR stimulation *in vitro* when atRA was present.
We hypothesize, treatment with atRA abrogates functional and phenotypic plasticity of myeloid cells. Therefore, timing of atRA administration should be carefully considered in therapeutic vaccination protocols to prolong a TLR-induced pro-inflammatory myeloid phenotype and to reduce immune suppression mediated by MDSCs.

P.B1.08.04
Sensitive identification and advanced profiling of neoantigen-specific T cells in cancer patients

S. Bobisse[1], *R. Genolet*[1], *B. J. Stevenson*[2], ***V. Bianchi***[1], *V. Zoete*[2], *D. Gfeller*[1], *L. Kandalaft*[1], *G. Coukos*[1], *A. Harari*[1];
[1]*Ludwig Institute for Cancer Research, Lausanne, Switzerland,* [2]*Swiss Institute of Bioinformatics, Lausanne, Switzerland.*

Neoantigens arise from tumor nonsynonymous somatic mutations and can generate immunogenic peptides (neo-epitopes) bound to HLA molecules. Mounting evidence suggests that neoantigens represent ideal tumor-specific candidates to target with personalized T-cell mediated immunotherapies or vaccines and several studies indeed demonstrated their clinical relevance. However, feasibility in low mutational load tumor types such as ovarian cancer remains unknown. Using highly sensitive assays to detect and profile neo-epitope specific circulating and tumor-infiltrating (TILs) $CD8^+$ T cells allowed prompt identification of oligoclonal and polyfunctional such cells from most immunotherapy-naïve patients with advanced epithelial ovarian cancer. Neo-epitope recognition was discordant between circulating T cells and TILs, and was more likely to be found among TILs, which displayed higher functional avidity and unique TCRs with higher predicted affinity than their blood counterparts. Of interest, the relative functional avidity of neoepitope-specific TILs correlated significantly with their intratumoral frequency. Our results imply that identification of neo-epitope specific $CD8^+$ T cells is achievable even in tumors with relatively low number of somatic mutations, and neo-epitope validation in TILs extends opportunities for mutanome-based personalized immunotherapies to such tumors.

P.B1.08.05
HLA ligandomics feeds a pipeline of soluble T cell receptor-based immunotherapies

F. Capuano, *G. Mommen, R. Carreira, D. Lowne, M. Cundell, A. Powlesland;*
Immunocore Ltd, Abingdon, United Kingdom.

Introduction: HLA complexes on cell surfaces can present cancer-associated peptides to the immune system, representing valuable targets for cancer immunotherapy. Immunocore identifies tumour-specific HLA peptides, generates peptide-specific T cell clones and engineers T cell receptors (TCRs) into potent soluble immunotherapeutics.
Materials and Methods: Immortalized cancer cells and tumour tissues are homogenised in lysis buffer and HLA complexes captured from the supernatant by affinity purification using HLA-restriction specific antibodies. HLA peptides eluted under acidic conditions are separated by reverse phase liquid chromatography and analysed by mass spectrometry. Fully validated target peptides are used to clonally expand HLA-peptide specific T cells. Isolated TCRs are engineered into soluble molecules (mTCRs) whose affinity towards target peptide:HLA is enhanced using phage display technology.
Results: Our HLA peptidomic workflow combines novel biochemical techniques with high resolution mass spectrometry, data integration from multiple instruments and multiple search algorithms to maximise the depth of the HLA ligandome (up to 400,000 unique HLA-A*02 peptides identified). Our immune-activating therapeutics (ImmTAC™) generated by coupling high-affinity mTCRs with an anti-CD3 scFv domain redirect polyclonal T cell responses toward cancer cells. Our current lead candidate, IMCgp100, has entered pivotal monotherapy trials for treatment of patients with metastatic uveal melanoma. Additionally, Immunocore in collaboration with MedImmune/AstraZeneca is conducting a trial in cutaneous melanoma patients exploring the combination of IMCgp100 with checkpoint inhibition, including Imfinzi® (durvalumab) and tremelimumab.
Conclusions: Our peptidomics workflow enables the identification of HLA-presented peptides to support the successful design of TCR-based immunotherapeutics for cancer treatment.

P.B1.08.06
ERAP1 controls the engagement of human NK cell receptor KIR3DL1 by its specific ligand HLA-B51

M. Compagnone[1], *V. D'Alicandro*[1], *P. Guasp*[2], *L. Cifaldi*[1], *P. Romania*[1], *G. Zicchedda*[1], *V. Lucarini*[1], *O. Melaiu*[1], *D. Pende*[3], *J. A. López de Castro*[2], *D. Fruci*[1];
[1]*Bambino Gesù Children's Hospital, IRCCS, Rome, Italy,* [2]*Centro de Biología Molecular Severo Ochoa, CSIS, Madrid, Spain,* [3]*IRCCS AOU San martino-IST, Genoa, Italy.*

The activity of natural killer (NK) cells is tightly regulated by inhibitory and activating receptors. Inhibitory killer immunoglobulin-like receptors (iKIRs) survey the surface of target cells by monitoring the expression of HLA class I (HLA-I) molecules. The interaction of iKIRs with HLA-I molecules is sensitive to the sequence of peptides bound to HLA-I molecules, suggesting that NK-cell activation may be regulated by a change in the repertoire of peptide. Recently, we have demonstrated that genetic or pharmacological inhibition of ERAP1, a key component of the antigen processing, on tumor cells perturbs the engagement of NK cell inhibitory receptors by their specific ligands, enhancing the NK-mediated killing. These results indicate that modulation of ERAP1 can be exploited as a novel tool to improve the efficacy of NK-based approaches for cancer immunotherapy.
To identify the HLA-I/KIR combinations most affected by ERAP1 inhibition, HLA-I-negative 721.221 cells transfected with a panel of KIR ligands (HLA-A2, -B51, -Cw3, -Cw4 and -Cw7) were stably silenced for ERAP1 expression and tested for their ability to induce NK-cell degranulation. HLA-I surface expression did not substantially change following ERAP1 inhibition. Conversely, CD107a expression was significantly upregulated on NK cells following stimulation with ERAP1-deficient 721.221-B51 cells as compared with control cells. This increase was particularly evident in the NK cell subset expressing the single KIR3DL1, and directly related to the functional level of this receptor. Overall, these results identify KIR3DL1/HLA-B51 as one of the most sensitive combinations to ERAP1 inhibition rendering tumor cells more susceptible to NK-mediated killing.

P.B1.08.07

Role of CD27/CD70 deficiency in the cell-mediated immune control of the Epstein Barr virus

***Y. Deng**, B. Chatterjee, C. Münz;*
Experimental Immunology, Zurich, Switzerland.

Introduction: Epstein Barr virus (EBV) is one of the most successful pathogens in the human population, persistently infecting more than 90% of adult individuals. It was discovered as the first human tumor virus contributes to 1-2% of all cancers in the world. Primary immunodeficiencies that predispose for EBV induced tumors and uncontrolled virus infection, have identified molecules in the differentiation, co-stimulation and effector function of cytotoxic lymphocytes. In this project, we are assessing CD27 immunodeficiency in EBV specific immune control and investigating which immune compartment(s) are compromised in the absence of CD27/CD70 co-stimulatory signal.
Material and Methods: To mimic the CD27 deficiency, anti-CD27 blocking and depletion antibodies were used to study the function of this pathway in EBV infection using T cell proliferation and killing assays *in vitro*. By reconstituting human immune system in mouse, the human-tropic EBV infection was able to be modeled *in vivo*. Conditions with/without CD27 injection were compared in terms of the EBV viral load and tumor incidence. In addition, the functionality of T and NK cells and cytokine production were monitored by flow cytometer.
Results: An impaired proliferation of T cells was observed due to the blocking effect of CD27 antibodies after 7 days of incubation. In vivo study showed depletion of $CD27^+$ cells in mice led to significant higher EBV viral loads and a slightly increased incidence of tumorigenesis
Conclusions: Overall, $CD27^+$ cells play a protective role in mediating EBV associated immune responses in humanized mice.

P.B1.08.08

Combination of Trastuzumab and Pertuzumab binding site mimotopes together with anti-PD1 immune checkpoint as a novel anti-Her-2/neu multi-level vaccine

*J. Tobias[1], **M. Drinic**[1], K. Baier[1], K. Ambroz[1], C. C. Zielinski[2], U. Wiedermann[1];*
[1]Institute of Specific Prophylaxis and Tropical Medicine, Center for Pathophysiology, Infectiology and Immunology, Medical University Vienna, Vienna, Austria, [2]Division of Oncology, General Hospital Vienna,, Vienna, Austria.

Extracellular subdomains of Her-2/neu is overexpressed in 20-25% of breast and gastric cancers. Combination of the mAbs Trastuzumab and Pertuzumab has synergistically resulted in a significant improvement in clinical outcomes of patients with Her-2/neu-positive metastatic breast cancer. However active immunotherapy, unlike application of mAbs, provides advantages like induction of humoral, cellular and memory responses and anti-tumor activity. The first generation of our B cell multi-peptide anti-Her-2/neu vaccine, containing three single peptides conjugated to virosomes, was recently improved to its second generation by fusing the peptides into a hybrid peptide (P467) which after conjugation to CRM197 and together with the adjuvant Montanide led to induction of strong humoral and Th1-biased cellular responses with antitumor activity. To broaden the number of biologically active epitopes in the vaccine, its third generation has recently been developed by including the binding site epitopes (mimotopes) of Trastuzumab and Pertuzumab, which in combination with P467 have shown to induce polyclonal humoral and cellular responses. Combinational therapy of cancer vaccines and immune checkpoint inhibitors has been suggested to synergistically enhance antitumor immune responses. When immunizing mice with P467-CRM-Montanide combined with anti-mouse PD-1 mAb vaccination, we have seen generally elevated cellular responses compared to unvaccinated mice. The third generation of our anti-Her2/neu vaccine combined with an immune checkpoint inhibitor is now being evaluated *in vivo* and may result in an effective novel multi-level vaccine against Her-2/neu overexpressing cancer entities. The study is granted by Imugene Limited, Australia, and Medical University of Vienna.

P.B1.08.09

LIGHT/LTβR signaling regulates self-renewal and differentiation of hematopoietic and leukemia stem cells

***S. Hoepner**[1], R. Radpour[1], M. Amrein[1], C. Riether[1], G. Baerlocher[2], A. Ochsenbein[1];*
[1]Inselspital, Bern University Hospital/Dept. Medical Oncology/Tumor Immunology, DBMR, University of Bern, Bern, Switzerland, [2]Inselspital, Bern University Hospital, Department of Hematology, Bern, Switzerland.

Introduction: Hematopoietic stem cells (HSC) are responsible to replenish all blood cell lineages. The balance between self-renewal, proliferation and quiescence is tightly regulated to ensure the maintenance of the stem cell pool but also guarantee rapid adaptation in response to hematopoietic stress. Likewise, the balance of self-renewal and differentiation is critical in the pathology of hematopoietic malignancies. Here we show that lymphotoxin-beta receptor (LTβR), a member of the TNF superfamily, and its ligand TNFSF14 (LIGHT) play an important role in HSC and leukemic stem cell (LSC) regulation. LIGHT/LTβR signaling maintains stem cell quiescence, promotes symmetric division and thereby contributes to HSC and LSC self-renewal.
Material and Methods: To study LIGHT/LTβR signaling, we performed serial competitive repopulation assays by using hematopoietic stem and progenitor cells (HSPCs) from *Ltbr* KO, *Light* KO and C57/BL6 (WT) mice and analyzed cell cycle activity, cell viability and cell division. In a second approach we analyzed LTβR signaling in HSCs after induction of genotoxic stress. Therefore, 5-Fluoracil (5-FU), a therapeutic agent which is known to activate HSCs, was administered into *Ltbr* KO and WT mice. Moreover, we investigated LIGHT/LTβR signaling in human HSPCs.
Results: *Ltbr* deficiency on murine HSCs and LSCs resulted in enhanced proliferation, asymmetric division and a reduced stem cell pool. LTβR signaling was induced by LIGHT in an autocrine manner. Moreover, *LTBR* deficiency affected the stemness of human HSPCs.
Conclusion: LIGHT/LTβR signaling is a novel player in HSC and LSC self-renewal, which potentially provide a new strategy to eliminate LSCs.

P.B1.08.10

Pancreatic and colon cancer stem cells evade NK cell effector function through PCNA-NKp44 interaction

***J. D. Malaer**, P. A. Mathew;*
UNT Health Science Center, Fort Worth, United States.

Introduction: NK cells participate in the innate immune response against infection and cancer without prior sensitization. NK cell function depends on a balance of signals transmitted from activating and inhibitory receptors interacting with ligands on the surface of cells. Cancer cells may evade NK-mediated killing by expressing ligands for inhibitory receptors. Proliferating cell nuclear antigen (PCNA) associates with HLA I and forms the inhibitory ligand for NKp44, resulting in the inhibition of NK function. Cancer stem cells (CSC), a unique subset of tumor cells, possess a stem-cell-like phenotype and are thought to facilitate metastasis by escaping NK cell effector function.
Materials and Methods: Pancreatic (Panc-1) and colon (HCT 116) cancer cells were labeled with antibodies against PCNA, CD44, and CD133 and surface expression was determined by flow cytometry. Cells were labeled and sorted for surface PCNA expression via FACS; NANOG, SOX2, and Oct-4 were analyzed by qRT-PCR from sorted cells. Target cells were blocked with anti-PCNA or control antibodies and a chromium release killing assay was performed.
Results: In both cell lines, cell surface PCNA is associated with co-expression of CD44 and CD133 as well as increased CSC transcription factor expression. Blocking the interaction of NKp44 and PCNA enhanced the specific lysis of cells by NK cells.
Conclusions: Collectively these data demonstrate that surface PCNA is a marker of pancreatic and colon CSC. Our research implicates that blocking NKp44-PCNA interaction may provide a novel immunotherapeutic target for pancreatic and colon cancer stem cells and prevent metastasis.

P.B1.08.11

Neoadjuvant therapy with attenuated Salmonella improves outcome of dacarbazine treated melanoma bearing mice

*S. Chilibroste, A. E. Mónaco, M. Vola, C. I. Agorio, J. A. Chabalgoity, **M. Moreno**;*
Universidad de la República, Montevideo, Uruguay.

Melanoma is a severe form of skin cancer with high incidence rate. After 30 years of use, d*acarbazine* (DTIC)-based chemotherapy continues to be the standard of care for most patients with metastatic melanoma. In this work, we evaluate the potential of *Salmonella enterica* serovar Typhimurim, LVR01 (*aroC*), as neoadjuvant therapy in melanoma-bearing mice undergoing chemotherapy. C57BL/6 mice were subcutaneously inoculated with B16F1 melanoma cells. When tumors were palpable, *S*. Typhimurium LVR01 ($1x10^6$ CFU) was intratumorally injected. At the following day, chemotherapy treatment consisting in daily intraperitoneally application of 150 mg/kg/doses DTIC was started and continued for 3 days. Neoadjuvant LVR01 administration in chemotherapy-treated mice retarded tumor growth and prolonged overall survival compared to control and monotherapy-treated animals. Importantly, Salmonella treatment was well tolerated by mice undergoing chemotherapy, with less than 10% of weight loss. This combined approach increased expressions of ccl2, ccl5, cxcl9 and cxcl10 mRNA levels in the tumor microenviroment, accompanied by tumor infiltrating neutrophils and cytotoxic lymphocytes with augmented activated status. Chemotherapy therapy induced a drastic reduction of secondary lymphoid organ size. Salmonella neoadjuvant treatment partially rescued absolute cell numbers in these compartments, and activated effector NK and CD8 T cells. In conclusion, *Salmonella* immunotherapy could be safely used in individuals under chemotherapy treatment. This therapeutic approach induces activation of cytotoxic lymphocytes, resulting in longer survival. The use of attenuated *Salmonella* as a non-specific active immunotherapy in combination with standard chemotherapy could be considered as an interesting therapeutic strategy with close clinical application for patients with melanoma.

P.B1.08.12

Anti-CD137 antibodies enhance Daratumumab efficacy against multiple myeloma increasing ADCC effect in an immunodeficient mouse model reconstituted with human NK cells

M. C. Ochoa[1,2,3], *E. Perez-Ruiz*[4], *A. Zabaleta*[5,2,3], *P. Berraondo*[6,2,3], *L. Minute*[6,2], *I. Rodriguez*[1], *A. López*[1,3], *S. Garasa*[6], *A. Azpilikueta*[6], *C. Molina*[6], *M. F. Sanmamed*[6], *A. Muntasell*[7], *B. Paiva*[6], *J. San Miguel*[1,3], *M. Lopez-Botet*[7,8], *I. Melero*[1,6,3];
[1]*Clínica Universidad de Navarra, Pamplona, Spain,* [2]*Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Pamplona, Spain,* [3]*Navarra Institute for Health Research (IDISNA), Pamplona, Spain,* [4]*Division of Medical Oncology, Hospital Costa del Sol, Marbella, Spain,* [5]*Centro de Investigacion Medica Aplicada (CIMA), Pamplona, Spain,* [6]*Centro de Investigación Médica Aplicada (CIMA), Pamplona, Spain,* [7]*Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain,* [8]*University Pompeu Fabra (UPF), Barcelona, Spain.*

Daratumumab is an anti-CD38 mAb approved for multiple myeloma treatment. Antibody-dependent cellular cytotoxicity (ADCC) mediated by NK cells and macrophages is one of the mechanisms of action proposed for this drug. Preclinical data have shown that the anti-CD137 agonist antibody Urelumab increases NK-mediated ADCC effect exerted by other mAbs used in oncology, such as Rituximab, Cetuximab and Trastuzumab, and clinical trials combining Urelumab and Rituximab or Cetuximab are ongoing. Freshly isolated human NK cells from healthy volunteers do not express CD137 on the membrane, but CD137 is induced when the NK cells are co-cultured with a Daratumumab-coated $CD38^+$ multiple myeloma-derived cell lines (MM1S or KMS28BM). Moreover, NK cells from multiple myeloma patient bone marrows treated *ex-vivo* with Daratumumab also up-regulate CD137 expression. Daratumumab addition to co-cultures of NK cells and MM1S or KMS28BM increases tumor cell death. However, cytotoxicity is not increased by the addition of Urelumab either in short-term (4h) or medium-term (18h) experiments. To study if Urelumab increases Daratumumab-mediated ADCC activity *in vivo,* we set up a tumor model based on the i.v. administration of a luciferase-transfected MM1S cell line, human NK cells and Daratumumab to immuno-deficient NSG mice. In this model, i.v. administration of Urelumab 24h after Daratumumab retarded the tumor growth and prolonged mice survival. Financial sources: Worldwide Cancer Research Foundation (15-1146); Fundación Española contra el Cáncer (GCB15152947MELE). Becas de Formación e Innovación. Junta de Andalucía

P.B1.08.13

The Wiskott-Aldrich syndrome protein regulates antigen processing and presentation by dendritic cells to activate cytotoxic T cells

M. Oliveira[1], *M. Baptista*[2], *L. Westerberg*[1];
[1]*Karolinska Institutet, Solna, Sweden,* [2]*Institute for Virology and Immunobiology, Würzburg, Germany.*

One promising cancer therapeutic today is to teach the patient's own immune system to kill tumor cells. Specialized dendritic cells (DCs) can take up exogenous antigen, such as tumor antigens, and present peptides on MHC class I molecules to activate $CD8^+$ cytotoxic T cells in a process called cross-presentation. Here, we examined the role of the actin regulator Wiskott-Aldrich syndrome protein (WASp) in antigen sorting in DCs. We found that specific deletion of WASp in DCs led to marked expansion of $CD8^+$ T cells. Mechanistically, we found that WASp-deficient DCs induced increased cross-presentation to $CD8^+$ T cells by activating Rac2 that maintained a near neutral pH of phagosomes. This allowed escape of antigen to the cytosol and loading on MHC class I molecules. To address if WASp directly reduced cross-presentation in DCs, we placed back WASp in bone marrow-derived DCs from WASp-deficient mice using Amaxa transfection. WASp-deficient DCs transfected with wildtype WASp induced decreased proliferation of $CD8^+$ T cells, suggesting that antigen sorting is regulated by WASp. To address if we could use our findings to target antigen sorting in wildtype DCs, we used small molecule inhibitors for actin regulators such as WASp. We have identified one inhibitor that induced increased cross-presentation by wildtype DCs. We treated DCs with this inhibitor during antigen sorting *ex vivo* and injected the DCs into mice. We found that inhibitor-treated DCs induced higher proliferation of antigen-specific $CD8^+$ T cells *in vivo*. Our data suggests that direct target of actin regulators may enhance DC-mediated immunotherapy.

P.B1.08.14

Using tumour origins to identify peptide vaccine targets in two independent contagious cancers

R. Owen[1], *A. Gastaldello*[1], *S. Ramarathinam*[2], *A. Bailey*[1], *P. Skipp*[1], *T. Elliott*[1], *A. W. Purcell*[2], *H. V. Siddle*[1];
[1]*University of Southampton, Southampton, United Kingdom,* [2]*Monash University, Melbourne, Australia.*

The Tasmanian devil harbours two distinct transmissible cancers, Devil facial tumour (DFT) 1 and 2. Both are spread by transmission of cells between individuals, threatening a vulnerable species. As these cancers are allografts, Major Histocompatibility Complex (MHC) and bound peptides are key to developing vaccine strategies. DFT1 originated in a Schwann cell, but the cellular origin of DFT2 is unknown. Here we identify the cellular origin of DFT2 and characterise the immunopeptidomes of DFT1 and DFT2 to identify antigenic peptides. Peptides from MHC class I were isolated in triplicate for each cell line using a devil anti-B_2m antibody. Mass spectrometry was used to sequence peptides and generate whole cell proteomes for DFT1-IFNγ, DFT2 and devil fibroblast cell lines. Peptides were identified by searching spectra against custom Tasmanian devil databases using PEAKS. Gene ontology analysis was performed. DFT2 and DFT1 are enriched for similar nervous system processes. DFT2 expresses proteins associated with glial development and myelin components. Between 2243 and 6373 potential MHC class I peptides were identified for each cell line with a length preference for 9mers. We identified 61 and 55 peptides unique to DFT1-IFNγ and DFT2. The immunopeptidomes of two contagious cancers will be used for vaccine design and to identify the binding motifs of MHC alleles expressed by DFT1 and DFT2. Our data suggests that DFT2 is of a myelin competent glial cell lineage and bears remarkable resemblance to DFT1, a finding significant for both vaccine design and fundamental understanding of transmissible cancers. The Leverhulme Trust (RPG-2015-203)

P.B1.08.15

Development of a novel *in vitro* screening method for cancer immunotherapy using genetically modified NK-92 cells: Dissection of NK cell-tumor cell interactions

D. Ozkazanc Unsal[1,2], *E. Celik*[1,2], *M. Chrobok*[3,4], *B. Erman*[1,2], *E. Alici*[3,4], *A. D. Duru*[3], *T. Sutlu*[1,2];
[1]*Nanotechnology Research and Application Center, Sabancı University, İstanbul, Turkey,* [2]*Faculty of Engineering and Natural Sciences, Sabancı University, İstanbul, Turkey,* [3]*NSU Cell Therapy Institute, Nova Southeastern University, Fort Lauderdale, United States,* [4]*Center for Hematology and Regenerative Medicine, Karolinska Institutet, Stockholm, Sweden.*

NK cell-mediated lysis relies on a balance between several activating and inhibitory receptors that either promote or dampen the killing upon receptor-ligand interactions. Here, we propose to develop a screening method based on the genetic modification of NK cells upregulating a single receptor at a time. As both the character of the tumor cell population and phenotypic status of NK cells differ among patients, such a tool will be instrumental in developing patient-tailored cancer immunotherapy approaches.
Genes coding for 20 different NK cell surface receptors were lentiviral vector backbones for genetic modification of NK-92 cells. New cell lines, each overexpressing a specific receptor, were subjected to phenotyping and assessment of effector functions. The use of a colorimetric substrate for Granzyme B activity allowed us to optimize an easy-to-use screening tool to identify targets of NK cell-tumor interactions on a color-based fashion. Degranulation against K562 cell line showed significantly higher cytotoxicity especially in CRACC, DNAM-1, NKG2D transduced NK-92 cells compared to wildtype and backbone controls. Lentiviral genetic modifications did not disrupt or hamper cytotoxicity of NK-92 cells but rather induced enhaced cytolysis against specific tumor ligands.
A novel *in vitro* screening tool can be established on genetically modified NK-92 cells expressing different activating receptors. It has the potential to be a standardized protocol to identify patient-specific targets for NK cell-based cancer immunotherapy.
*This research is supported by the Scientific and Technological Research Council of Turkey (TUBITAK) grant 114Z861.

P.B1.08.16

Evaluation of intravenous immunoglobulin use analyzed in relation to diagnostic evidence levels

D. Garcia-Cuesta[1], *M. Vilches-Moreno*[1], *A. Salguero-Olid*[2], *M. San Jose*[1], ***A. Sampalo***[1];
[1]*UGC Hematology, Immunology and Genetics. Hospital Puerta del Mar., Cádiz, Spain,* [2]*UGC Hospital Pharmacy. Hospital Puerta del Mar., Cádiz, Spain.*

Aim: To evaluate clinical indication of intravenous immunoglobulin (IGIV) in the last year in our hospital in relation of based guideline of immunoglobulin Therapy. Evidence category and strength of recommendation was indicated.
Method: IGIV treatment was prescribed for 145 patients (76 male/ 69 female; median age 43± (range 5 moths - 90 years). Clinical data, evidence category (EC) and strength of recommendation (SC) for indications. Data was evaluated in relation to 2016 update of the Consensus Document of the American Academy of Allergy, Asthma and Immunology.
Results: IGIV were prescribed in 71 patients (49%) for **indications definitively benefit** : 19 ,Primary immune deficiencies(IIb/B) 27 Immune thrombocytopenic purpura (Ia/A), 12 Guillain Barré Syndrome (IIb/B), 4 Kawasaki disease (Ia/A), 6 Chronic desmyelinizating polyneuropathy (1a/A), 3 Multifocal motor neuropathy. IVIG were prescribed in 58 patients (40%) for **indications probably benefit** : 14 secondary immune deficiencies (IV/D) 21 Miastenia gravis (Ib/B, 1 dertomyositis (IIa/B) (1), 1 relapsing remitting multiple sclerosis (Ia/A), 8 Intractable childhood epilepsy (1a/B), 1 acute disseminated encephalomyelitis, 2 post hematopoietic cell transplantation (IV/B), 7 antibody mediated rejection (IV, D), 3 highly sensitized patients for renal transplantation (IV,D). IVIG were prescribed for **indications that may provide benefit** in 8 cases (5.5%): 2 autoimmune blistering skin disease (1a/A), 3 severe resistant atopic dermatitis, 1 severe asthma. IVIG were prescribed for **indications with no clinical evidence of benefit** in 8 (5.5%) patients.
Conclusions: Data demonstrate an appropriate use of immunoglobulin. Uses in which no clear effective benefit was demonstrated might be avoided.

P.B1.08.17

Acute pharmacologic degradation of a stable antigen enhances its direct presentation on MHC class I molecules

*S. C. Moser[1], J. S. Voerman[1], D. L. Buckley[2], G. E. Winter[3], **C. Schliehe**[1];*
[1]Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands, [2]Department for Medical Oncology, Dana-Farber Cancer Institute, Boston, United States, [3]CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria.

Bifunctional degraders, also referred to as proteolysis-targeting chimeras (PROTACs), are a recently developed class of small molecules. They were designed to specifically target endogenous proteins for ubiquitin/proteasome-dependent degradation and to thereby interfere with pathological mechanisms of diseases, including cancer. In this study, we hypothesized that this process of acute pharmacologic protein degradation might increase the direct MHC class I presentation of degraded targets. By studying this question, we contribute to an ongoing discussion about the origin of peptides feeding the MHC class I presentation pathway. Two scenarios have been postulated: peptides can either be derived from homeostatic turnover of mature proteins and/or from short-lived defective ribosomal products (DRiPs), but currently, it is still unclear to what ratio and efficiency both pathways contribute to the overall MHC class I presentation. We therefore generated the intrinsically stable model antigen GFP-S8L-F12 that was susceptible to acute pharmacologic degradation *via* the previously described degradation tag (dTAG) system. Using different murine cell lines, we show here that the bifunctional molecule dTAG-7 induced rapid proteasome-dependent degradation of GFP-S8L-F12 and simultaneously increased its direct presentation on MHC class I molecules. Using a doxycycline-inducible setting, we could further show that the stable, mature antigen was the major source of peptides presented in our system. This study is, to our knowledge, the first to investigate targeted pharmacologic protein degradation in the context of antigen presentation and our data point toward future applications by strategically combining therapies using bifunctional degraders with their stimulating effect on direct MHC class I presentation.

P.B1.08.18

Antitumor activity of Gastropodan hemocyanins in murine model of colon carcinoma

*E. Stoyanova[1], I. Manoylov[1], N. Mihaylova[1], K. Idakieva[2], **A. I. Tchorbanov**[1];*
[1]Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria, [2]Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria.

Introduction: The hemocyanins (Hcs) are oligomeric copper-containing glycoproteins that function as oxygen carriers in the hemolymph of several molluscs and arthropods. Molluscan Hcs have been studied intensively for many years as very promising class of anti-cancer therapeutic, generating strong humoral and cellular immune response. A possible mechanism for their antitumor effect is the presence of cross-reactive epitopes between the carbohydrate content of the hemocyanin molecule and tumor-associated carbohydrate antigens, which are characteristic for different types of cancer. Materials and Methods: The Hcs were isolated from marine snail *Rapana thomasiana* (RtH) and the terrestrial snail *Helix pomatia* (HpH). Murine colon carcinoma cell line C-26 was used for animal administration and solid tumor establishment. Flow cytometry was performed for phenotyping of spleen and tumor suspensions and an apoptosis assay. The levels of cytokines and anti-C-26 antibodies were quantified by ELISA. Results: The Hcs exhibited strong *in vivo* anti-cancer and anti-proliferative effects in the developed murine model of colon carcinoma. We observed a significant increase of the spleens in non-treated C-26-bearing mice compared to Hcs treated. The immunization with RtH and HpH prolonged the survival of treated animals, improve humoral anti-cancer response and moderate the manifestation of C-26 carcinoma symptoms as tumor growth, splenomegaly and lung metastasis appearance. Conclusions: Hemocyanins are used so far for therapy of superficial bladder cancer and murine melanoma models. Our findings demonstrate a potential anti-cancer effect of hemocyanins on a murine model of colon carcinoma suggesting their use for immunotherapy of different types of cancer.

P.B1.08.19

Efficacy of generating immunogenic fast dendritic cells *in vitro* depend on the phenotype and early cytokines production by monocytes

*B. Pavlovic[1], **S. Tomic**[2], J. Djokic[3], D. Mihajlovic[4], D. Vucevic[4], M. Colic[2];*
[1]Pharmaceutical Faculty, University in Belgrade, Belgrade, Serbia, [2]Institute for the Application of Nuclear Energy, Belgrade, Serbia, [3]Laboratory for molecular microbiology, Institute for Molecular Genetics and Genetical Engineering, University in Belgrade, Belgrade, Serbia, [4]Medical Faculty of the Military Medical Academy, University of Defence, Belgrade, Serbia.

Cancer immunotherapy based on fast dendritic cells (fDC) is a promising and physiologically more relevant approach. However, not all donors respond well to fast DC protocol, leading to differentiation of potentially tolerogenic fDC, which may be deleterious for cancer treatment. Out of 10 donors tested in this work, we identified 4 whose monocytes do not generate immunogenic DC after 24h differentiation with granulocyte-macrophage colony stimulating factor and IL-4, followed by 48h stimulation with Poly (I:C). Non-responder fDC displayed high CD14 expression, low CD1a and costimulatory molecules expression, and low IL-12p70/TGF-β production ratio. Additionally, non-responder fDC displayed a weaker allostimulatory capacity, and a higher potential to induce alloreactive $CD4^+CD25^{hi}FoxP3^{hi}$ T regulatory cells, compared to responder fDC. These properties of non-responder fDC correlated positively with the expression of CD16 and the production of IL-6 and IL-1β early in cultures, and negatively with the expression of CD36 and CD69 and IL-8 production by the precursor monocytes. The blocking IL-6R with tocilizumab during the differentiation of non-responder monocytes into fDC, improved significantly the immunogenic phenotype and functions of fDC, and diminished their capacity to induce Tregs due to down-regulated expression of PD-1L and ILT-3. Our results stress out the importance of careful monitoring of cell cultures for identification of potential non-responder monocytes, and suggest timely actions toward increasing the immunogenic potential of fDC for cancer immunotherapy.

P.B1.09 Tumor vaccination principles and Immunotherapy - Part 9

P.B1.09.01

Cancer vaccines: Designing synthetic long peptides to improve presentation of selected class I and class II T cell epitopes by dendritic cells.

***C. Rabu**[1,2,3], L. Rangan[4,5], O. Adotevi[4,5], N. Labarrière[1,3], F. Lang[1,2,3];*
[1]INSERM U1232, Nantes, France, [2]Université de Nantes, Nantes, France, [3]Labex IGO, Nantes, France, [4]INSERM U1098, Besancon, France, [5]EFS Bourgogne France-Comté, Besancon, France.

Numerous class I and II T cell epitopes from tumor antigens have been characterized and exploited in vaccination protocols. Relying solely on short class I epitopes failed to elicit strong clinical responses while the simultaneous recruitment of CD4 T cells greatly enhanced vaccine efficacy. Thus, recent trials use synthetic long peptides (SLP) to activate both CD4 and CD8 T cells. The long peptides used consisted mainly in a mix of extended class I epitopes requiring DC internalisation for presentation and containing undefined class II epitopes. However, on native tumor antigens, the immunodominant epitopes can be either separated by hundreds of amino acids or on the contrary, overlap which could impair their processing efficiency.
Our strategy is to bring together a defined CD4 class II epitope to a defined CD8 class I epitope, joining them with a cathepsin-sensitive linker to increase its intracellular processing by DC.
As model antigens, we used MELOE-1 and MelanA/Mart1 widely expressed in melanoma and containing immunodominant epitopes. We tested a wide serie of short linkers (4 to 6 aa) selected for potential cathepsin sensitivity. We observed up to hundred fold differences in cross-presentation efficiency while classical class II presentation was less affected. We also confirmed that optimized SLP are more efficient than native antigen to expand specific CD8 T lymphocytes from whole PBMC in vitro. In a humanized mouse model, we show that immunization with optimized SLP tends to increase crosspresentation in vivo.
The selection of an optimized linker in the design of SLP vaccines should significantly increase the efficacy of therapeutic vaccination in cancer patients.

P.B1.09.02

Listeria based nanovaccines as therapeutic vaccines in cancer

***C. Alvarez-Dominguez**[1], H. Teran-Navarro[1], R. Calderon-Gonzalez[1], D. Salcines-Cuevas[1], J. Freire[2], J. Gomez-Roman[2], A. Garcia-Castaño[2], F. Rivera-Herrera[2], S. Yañez-Diaz[2];*
[1]Instituto de Investigación Marqués de Valdecilla, Santander, Spain, [2]Hospital Universitario Marqués de Valdecilla, Santander, Spain.

Dendritic cell-based (DC-based) vaccines are promising immunotherapies for cancer. However, several factors, such as the lack of efficient targeted delivery and the sources and types of DCs, have limited the efficacy of DCs and their clinical potential. We propose an alternative nanotechnology-based vaccine platform with antibacterial prophylactic abilities that uses gold glyconanoparticles coupled to listeriolysin O 91-99 peptide (GNP-LLO91-99), which acts as a novel adjuvant for cancer therapy as well as therapeutic vaccine for cutaneous melanoma acting as a novel immunotherapy. GNP-LLO91-99, exhibited dual anti-tumour activities, namely, the inhibition of tumour migration and growth and adjuvant activity for recruiting and activating DCs, including those from melanoma patients. GNP-LLO91-99 nanoparticles caused tumour apoptosis and induced antigen- and melanoma-specific cytotoxic Th1 responses ($P \leq 0.5$). They also cause tumour complete remission and survival improvement. GNP-LLO_{91-99} nanovaccines presented superior tumour rejection and survival benefits, when combined with anti-PD-1 or anti-CTLA-4 checkpoint inhibitors, predicting an improvement of these immunotherapies action. Studies with monocyte-derived DCs of patients with stage IIIB melanoma confirmed the ability of GNP-LLO_{91-99} nanovaccines to complement the action of check point inhibitors, not only reducing cell-death markers expression on DCs, but also potentiating DC antigen-presentation and production of Th1-Th12 cytokines. We propose that GNP-LLO_{91-99} nanovaccines function as immune stimulators and immune effectors and serve as safe cancer therapies, alone or in combination with other immunotherapies.

P.B1.09.03

Functional study of tumor infiltrating lymphocytes in a breast cancer patient: an approach to personalized medicine

A. Aran[1], *M. Bernuz*[1], *V. Peg*[2], *C. Bernadó*[2], *E. Zamora*[2], *J. Soberino*[3], *J. Pérez*[3], *E. Holgado*[4], *J. Arribas*[2], *J. Cortés*[4,3,2], *M. Martí*[1];
[1]*Universitat Autònoma de Barcelona, Bellaterra, Spain,* [2]*Vall d'Hebron Institute of Oncology, Barcelona, Spain,* [3]*Instituto Oncológico Baselga - Quirón Hospital, Barcelona, Spain,* [4]*Ramón y Cajal University Hospital, Madrid, Spain.*

Breast cancer is the most common of women cancers. Triple-negative BC (TNBC), negative for estrogen and progesterone receptor and HER-2 genes, represent a clinical challenge because they do not respond to endocrine therapy or other targeted agents. Studying of tumor infiltrating lymphocytes (TILs) is a promising field because of their good correlation with patient survival, especially those with high CD8/Treg ratio.
We have studied TILs from a TNBC patient to characterize the immune response. TILs were obtained from a core biopsy that was cut in serial slices and cultured. Molecular and functional phenotype of TILs was studied by immunostaining of TILs, cytokine release and suppression assays, all analyzed by flow cytometry. TCRs from TILs, before and after expansions, have been sequenced in order to find if there are monoclonal expansions.
TILs were a mixture of populations in all cultures, different CD8/CD4 ratios were observed related with their location on the biopsy. This different distribution of CTLs and CD4 TILs also affected the immune mediators detected in the supernatant, i.e. higher presence of cytotoxicity-related proteins Granzyme B and IFNγ in cultures with CTLs dominance. No cytokine profile could be defined on cultures with predominant CD4 T cells. TILs were expanded in vitro and tested in standard regulation assays. Cultures with predominant CTLs showed less capacity of inhibiting alloreactive proliferation compared with CD4 T cell cultures.
We have observed a heterogeneous distribution of TILs in the biopsy that may be useful to select the appropriate T cells to design tailored approaches to TNBC treatment.

P.B1.09.04

Heterologous prime-boost vaccination protects from Epstein-Barr virus antigen-expressing lymphomas

J. Rühl[1], *C. Citterio*[1], *C. Leung*[2], *C. Münz*[1];
[1]*Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland,* [2]*Nuffield Department of Medicine, Ludwig Institute for Cancer Research, University of Oxford, Oxford, United Kingdom.*

Epstein-Barr virus (EBV) is a γ-herpesvirus that preferentially infects B cells and establishes life-long chronic infection in more than 90% of the adult human population worldwide. The virus-host balance is mainly sustained by T-cell responses, which are able to control the infection asymptomatically. However, when T-cell immune control fails, EBV is associated with a number of human malignancies such as Burkitt's lymphoma, Hodgkin's lymphoma and nasopharyngeal carcinoma. EBV is one of the predominant tumor viruses in humans, but so far no therapeutic or prophylactic vaccination against this transforming pathogen is available. We demonstrate that heterologous prime-boost vaccination with the nuclear antigen 1 of EBV (EBNA1) either targeted to the DEC205 receptor on dendritic cells or expressed from a recombinant modified vaccinia virus Ankara (MVA) vector improves priming of antigen-specific $CD4^+$ T-cell help. $CD4^+$ T-cell help supports the expansion and maintenance of EBNA1-specific $CD8^+$ T cells that are most efficiently primed by recombinant adenoviruses that encode EBNA1. These combined $CD4^+$ and $CD8^+$ T-cell responses protect from EBNA1-expressing T cell lymphomas and B cell lymphoproliferations that emerge spontaneously after EBNA1 expression. Especially the heterologous EBNA1-expressing adenovirus, boosted by EBNA1-encoding MVA vaccination demonstrated protection as prophylactic and therapeutic treatment of the respective lymphoma challenges. Therefore, we propose that such heterologous prime-boost vaccinations should be further explored for clinical development against EBV-associated malignancies as well as symptomatic primary EBV infection.

P.B1.09.05

Live cell imaging of lytic granule motility in anti-ErbB2 CAR NK cells and FcR NK cells plus Herceptin towards ErbB2+ breast cancer cells

N. Wotschel[1,2,3], *J. Eitler*[1,2,3], *T. Müller-Reichert*[4], *M. Gerlach*[5], *W. Wels*[6,7], *T. Tonn*[1,2,3], *H. G. Klingemann*[8];
[1]*Experimental Transfusion Medicine, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany,* [2]*Institute for Transfusion Medicine Dresden, German Red Cross Blood Donation Service North-East, Dresden, Germany,* [3]*German Cancer Consortium (DKTK), Partner Site Dresden, Dresden, Germany,* [4]*Structural Cell Biology Group, Experimental Center, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany,* [5]*Structural Cell Biology Group, Experimental Center, Medical Faculty Carl Gustav Carus, University of Technology, Dresden, Germany,* [6]*Institute for Tumor Biology and Experimental Therapy, Georg-Speyer-Haus, Frankfurt am Main, Germany,* [7]*German Cancer Consortium (DKTK), Partner Site Frankfurt/Mainz, Frankfurt am Main, Germany,* [8]*Nantkwest Inc., Culver City, United States.*

Upon encountering a susceptible target, NK cells mediate directed cytotoxicity by exocytosis of lytic effector molecules such as perforin and granzymes. The steps leading to NK cell granule exocytosis are highly regulated. Granule exocytosis is preceded by convergence of granules to the microtubule organizing center (MTOC) and subsequent polarization of the MTOC and granules to the immunological synapse (IS). In case of antibody-dependent cell-mediated cytotoxicity (ADCC), it has been shown that signaling through the Fc receptor is critical to polarize MTOC and granules to the IS with otherwise resistant targets. Here we used spinning disk confocal microscopy for live cell imaging to analyze granule-mediated NK cell cytotoxicity in ErbB2-targeted CAR expressing NK-92 cells (NK-92/5.28.z) and research-grade high affinity FcR expressing NK-92 cells plus Herceptin™ towards ErbB2-positive breast cancer cells (MDA-MB-453), which are resistant to parental NK-92. Interestingly, unmodified NK-92 cells in combination with MDA-MB-453 cells showed granule convergence to the MTOC, but failed to polarize MTOC and granules to the IS. In contrast, retargeting by either CAR or mAb/FcR towards the ErbB2 antigen on MDA-MB-453 enabled granule polarization to the IS resulting in highly effective cytotoxicity. Granule polarization was rapid in both the ErbB2 and high affinity FcR expressing NK-92 cells after cell-cell contact was initiated (3 and 9 minutes respectively). These observations suggest that retargeting of NK-92 cells by either transgenic CAR or high affinity FcR expression in combination with tumor-specific antibodies confers tumor cell lysis by enabling the otherwise impaired MTOC and granule polarization to the IS which resembles the physiological exocytosis cascade observed in naturally occurring ADCC.

P.B1.09.06

Activation of phosphorylation of STAT3 by farnesoid X receptor accelerates the migration of NSCLC cell induced by lung fibroblast

H. JIANG, *X. Liu;*
RENJI HOSPITAL, SCHOOL OF MEDICINE, SHANGHAI JIAO TONG UNIVERSITY, SHANGHAI, China.

Objective: We aimed to study that whether the bile acid nuclear receptor (FXR) played a role in the complex dynamic interaction between cancer associated fibroblasts (CAFs) and lung cancer cells. **Materials and methods:** Human lung cancer cells (A549 cells) were co-cultured with lung fibroblasts (HPF cells) for 48 hours in vitro. The expression of proteins, the migration abilities and the levels of cytokines were detected by western blot assay, the transwell migration assay and cytokines antibody arrays respectively. **Results:** Western blot results showed that the expression of FXR and STAT3 phosphorylation (at Tyr705) in the A549 cells increased in the co-culture system. The transwell migration assay showed that the cell migration ability in the A549 cells was also improved compared with the control group ($P<0.001$). Meanwhile, the levels of some cytokines such as TNF RII, IL-17B and RGM-B were significantly up-regulated in the co-culture supernatant ($P<0.05$). The levels of TNF RII, IL-17B and RGM-B increased 18.2, 11.3 and 8.7 times as high as the controls respectively. However, these effects could be reversed by silencing FXR with si-RNA in A549 cells ($P<0.05$). **Conclusion:** FXR accelerates to the migration of NSCLC induced by lung fibroblast-tumor cells interaction though STAT3 signaling pathway.

P.B1.09.07

Neutral sphingomyelinase 2 heightens anti-melanoma immune response and synergizes with immune checkpoint inhibitors

A. Montfort[1], *F. Bertrand*[1], *J. Rochotte*[1], *J. Gilhodes*[2], *T. Filleron*[2], *C. Imbert*[1], *M. Tosolini*[3], *C. Clarke*[4], *F. Dufour*[5], *O. Micheau*[5], *M. Record*[1], *P. Cordelier*[1], *S. Sylvente-Poirot*[1], *N. Therville*[1], *N. Andrieu-Abadie*[1], *T. Levade*[6], *Y. Hannun*[4], *H. Benoist*[6], *C. Colacios*[6], *B. Ségui*[6];
[1]*INSERM U1037, CRCT, Toulouse, France,* [2]*Institut Universitaire du Cancer (IUCT), Toulouse, France,* [3]*INSERM U1037, Toulouse, France,* [4]*Stony Brook University, Stony Brook, NY 11794, United States,* [5]*INSERM UMR866, Dijon, France,* [6]*INSERM U1037, CRCT, Université Toulouse III, Toulouse, France.*

Background: Neutral sphingomyelinase 2 (nSMase2) belongs to a network of sphingolipid-metabolizing enzymes. More specifically, it catalyses the hydrolysis of sphingomyelin into ceramide, a bioactive lipid considered as an anti-oncometabolite. **Results:** Gene expression analyses from melanoma tumours of the TCGA dataset revealed the gene coding for nSMase2, *SMPD3*, was expressed at lower levels in metastases as compared to primary masses. Moreover, high levels of the transcript coding for nSMase2 in tumour biopsies were associated with better overall survival for advanced melanoma patients. In the mouse B16 melanoma model, which displays low levels of nSMase2, overexpression of this enzyme did not affect cell growth under 2D or 3D culture conditions, however it decreased tumour growth *in vivo*. Surprisingly, nSMase2 overexpression increased the infiltration of tumours by CD8+ T cells and the nSMase2-dependent delayed tumour growth was abolished in CD8 KO mice. Mechanistically, increased nSMase2 activity led melanoma cells to secrete exosomes enriched for the immunogenic miR155, thus favouring dendritic cell activation and anti-melanoma CD8+ T cell responses *in vivo*. Finally, increased nSMase2 activity in tumours synergized with anti-PD-1 therapy to abolish melanoma growth *in vivo*. **Conclusion:** Our work highlights a new role for the sphingolipid metabolism in the modulation of the immune microenvironment of melanoma by malignant cells.

P.B1.09.08

Use of primary T cells for delivery of mRNA encoded immunotoxins

R. Eggers[1], *A. Philippi*[1,2], *M. O. Altmeyer*[1], *F. Breinig*[3], *M. J. Schmitt*[3];
[1]*Environmental Safety Group, Korea Institute of Science and Technology (KIST) Europe, 66123 Saarbrücken, Germany,* [2]*BioNTech AG, 55131 Mainz, Germany,* [3]*Department of Biosciences, Molecular and Cell Biology and Center of Human and Molecular Biology (ZHMB), Saarland University, 66123 Saarbrücken, Germany.*

In cancer therapy, immune cells can be used as delivery platform for highly toxic compounds like immunotoxins in order to reduce their side effects. Here we investigated the potential of human primary T cells to deliver immunotoxins. T cells were engineered by transient transfection with immunotoxin encoding mRNA. The recombinant toxin, secreted by T cells, was expected to cause an increased efficiency in adoptive T cell therapy. Therefore two *Pseudomonas exotoxin* A-based immunotoxin constructs (e23-PE38 and VEGF-PE38) were designed. *Ex vivo* activated, primary T cells were transfected with *in vitro* synthesized mRNA, coding for e23-PE38 and VEGF-PE38. Successful expression and secretion of the immunotoxin were shown by Westernblot and Sandwich-ELISA analyses. WST-1 assay revealed that immunotoxin expression impaired the viability of transfected T cells. Nevertheless, *in vitro* toxicity tests showed that immunotoxin expressing T cells were able to perform residual effector functions, mediated by HEA125xOKT3 bispecific antibody. An additional effect of the immunotoxin was not observed. It was possible to restore impaired viability and reduced proliferation of transfected T cells by expressing an attenuated version of VEGF-PE38. MALDI-TOF analysis revealed that the immunotoxin was partially translocated into the endoplasmic reticulum.
By expressing mRNA encoded immunotoxins in T cells, we were able to demonstrate the potential of T cells for mRNA-mediated drug delivery. Therefore mRNA mediated protein expression might be a promising tool for T cell mediated drug delivery.

P.B1.09.10

The effect of vitamin E on the function and frequency of myeloid-derived suppressor cells in an experimental breast cancer model

Y. Vojgani, *S. Habibi, J. Hadjati, M. Vojgani;*
Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of.

Background: Vitamin E has been shown to have strong anti-carcinogenic properties, including antioxidant and apoptotic characteristics, making it appealing candidate for cancer therapy. On the other hand, among the tumor immunosuppressive components, it has been shown myeloid derived suppressor cells (MDSCs) have remarkable ability to suppress anti-tumor immunity through multiple mechanisms. The aim of current study was to assess whether the alpha-tocopherol succinate can alleviate MDSCs-mediated immunosuppression in vitro and in an experimental breast cancer model. **Material and Methods:** After assessing the effect of α-tocopherol succinate on MDSC viability and gene expression in vitro, mice were challenged with $7x10^5$ 4T1 murine breast adenocarcinoma cell line. After 5 days, tumor-bearing mice were intraperitoneally injected with vitamin E (5mg/kg) or DMSO/Tween (vitamin E solvent) at one day interval for a total of five times. After isolation of MDSCs from the spleen and tumor tissue, MDSCs frequency, nitric oxide (NO) production and gene expression analysis were performed by flow cytometry and quantitative RT PCR respectively. **Results:** Based on our experiments, vitamin E diminished tumor growth rate in tumor bearing mice but it had no effect on the percentage of CD11b+ Gr-1+ MDSCs in the spleen and tumor tissues in tumor-bearing mice. Q-PCR showed that α-tocopherol succinate reduced iNOS, Arginase and indoleamine-pyrrole 2,3-dioxygenase (IDO) gene expression in isolated MDSCs. **Conclusion:** According to our data, vitamin E has not effect on MDSCs frequency in tumor bearing mice however it may modulate their functions through decreasing MDSC expression of immunosuppression-related genes *in vitro.*

P.B1.09.11

Unbiased identification of CD4⁺T-cell epitopes using novel MHC-based chimeric receptors.

J. Kisielow, *F. Obermair, M. Kopf;*
Institute of Molecular Health Sciences, ETH, Zürich, Switzerland.

αβT-cell receptors(TCRs) bind peptide-major histocompatibility complexes(pMHC) with low affinity, posing a considerable challenge for direct identification of αβT-cell cognate peptides (epitopes). Here, we describe a platform for the discovery of MHC class-II presented epitopes, based on screening of engineered reporter cells expressing novel pMHC-TCR (MCR) hybrid molecules carrying cDNA-derived peptides. This technology identifies natural epitopes of CD4 T-cells in an unbiased and efficient manner and allows detailed analysis of TCR cross-reactivity providing recognition patterns on top of discrete epitopes. We identify cognate peptides of virus- and tumor-specific T-cells in mouse disease models and present a proof-of-concept for human T-cells. Furthermore, we show that vaccination with a peptide naturally recognized by TILs can efficiently protect from tumor challenge. Thus, the MCR technology holds promise for basic research and clinical applications allowing personalized identification of T cell antigens in patients.

P.B1.09.12

Memory CD8 T cell inflation promotes tissue-residency

E. T. I. van der Gracht[1], *A. N. Yilmaz*[1], *S. van Duikeren*[1], *P. Klenerman*[2], *F. Koning*[1], *R. Arens*[1];
[1]*LUMC, Leiden, Netherlands,* [2]*University of Oxford, Oxford, United Kingdom.*

Memory T cell inflation is a phenomenon occurring upon infection with certain chronic viruses that is characterized by the maintenance of large populations of circulating antigen-specific memory CD8+ T cells with an effector-memory-like phenotype. Whether memory T cell inflation is related to the formation and maintenance of tissue-resident memory (TRM) T cells is not known. Here we studied the induction and maintenance of CD8+ TRM T cells upon immunization with adenoviral vectors modified to elicit memory T cell inflation. Mice immunized with different doses of adenovirus encoding full-length HPV E7 protein or the immunodominant epitope from E7 protein elicited E7-specific memory inflation in a dose-dependent manner. Interestingly, E7-specific CD8+ TRM T cells were generated and maintained for months in multiple organs after vaccination, and the numbers of these CD8+ TRM T cells associated with memory inflation. The vaccine-induced CD8+ T cell responses conferred long-term protection in a mouse model of HPV-induced carcinoma, and this protection depended on the development of CD8+ TRM T cells. Moreover, this formation of CD8+ TRM T cells could be enhanced by temporal targeting costimulatory interactions early after immunization. Together, these data suggest that the induction of tissue-residency is linked to the memory inflation, and can be enhanced by targeting costimulation.

P.B1.09.13

The *Taenia crassiceps*-derived immunomodulatory peptide GK-1 controls tumor growth by promoting tumor-specific CD8 T cell infiltration and effector function

N. Rodriguez Rodriguez[1,2], *J. Cervantes*[2], *F. Rosetti*[1], *J. C. Crispin*[1], *G. Fragoso*[2], *E. Sciutto*[2];
[1]*Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Mexico,* [2]*Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico.*

GK-1 is a synthetic 18 amino acid-peptide, originally derived from *Taenia crassiceps,* that has shown immunomodulatory properties in several immunization models. Our group and others demonstrated that GK-1 administration decreases tumor burden and metastasis, while improving survival in mouse models of cancer. Such a result was ascribed to GK-1 influence on antigen presenting cells (APC).
To further dissect the immunomodulatory properties of GK-1, T cell-deficient mice previously subcutaneously injected with ovalbumin (OVA)-expressing B16-F0 melanoma cells were weekly intravenously given GK-1. Contrary to results obtained with T-cell immunosufficient mice, where GK-1 demonstrated antitumor properties, administration of GK-1 neither prevented tumor development nor increased survival, indicating that T cells are required for GK-1 antitumor effect.
The capacity of GK-1 to promote tumor-specific T cell responses was evaluated by transferring OT-I $CD8^+$ T cells specific for OVA into mice that received B16-OVA cells. GK-1 significantly decreased tumor incidence and size compared to control individuals, regardless of co-administration of OVA. This was associated with increased numbers of tumor-infiltrating OT-I cells, and a higher proportion of cells able to proliferate and produce IFN-γ upon antigen encounter. Tumor-associated dendritic cells displayed elevated CD86 expression and OVA presentation. Interestingly, GK-1 administration to mice transferred with OVA-specific $CD4^+$ T lymphocytes was unable to reduce tumor development, despite in vitro results demonstrating that GK-1 indirectly stimulates $CD4^+$ cell, but not $CD8^+$ cell, proliferation by activating APC.
In conclusion, GK-1 reduces tumor establishment and growth. $CD8^+$ lymphocytes are necessary to mediate this effect, while $CD4^+$ cells probably act as relays transferring the GK-1-induced stimulation of APCs to $CD8^+$ lymphocytes.

P.B1.09.14

In situ delivery of allogeneic natural killer cell (NK) combined with Cetuximab in liver metastases of gastrointestinal carcinoma: A phase I clinical trial

O. Adotévi, Y. Godet, ***J. Galaine****, Z. Lakkis, I. Idirene, J. Certoux, M. Jary, R. Loyon, C. Laheurte, S. Kim, A. Dormoy, F. Pouthier, C. Barisien, F. Fein, P. Tiberghien, X. Pivot, S. Valmary-Degano, C. Ferrand, P. Morel, E. Delabrousse, C. Borg;*
UMR1098, BESANCON, France.

Despite successful introduction of NK-based cellular therapy in the treatment of myeloid leukemia, the potential use of NK alloreactivity in solid malignancies is still elusive.
We performed a phase I clinical trial to assess the safety and efficacy of *in situ* delivery of allogeneic NK cells combined with cetuximab in liver metastasis of gastrointestinal origin. The conditioning chemotherapy was administrated before the allogeneic NK cells injection via hepatic artery. Three escalating doses were tested (3.10^6, 8.10^6 and 12.10^6 NK cells/kg) following by a high-dose interleukin-2 (IL-2). Cetuximab was administered intravenously every week for 7 weeks. Nine patients with liver metastases of colorectal or pancreatic cancers were included, three per dose level. Hepatic artery injection was successfully performed in all patients with no report of dose-limiting toxicity. Two patients had febrile aplasia requiring a short-term antibiotherapy. Grade 3/4 anemia and thrombopenia were also observed related to the chemotherapy.

Objective clinical responses were documented in 3 patients and among them 2 occurred in patients injected with cell products harboring two KIR ligand mismatches and one in a patient with one KIR ligand mismatch. Immune monitoring revealed that most patients presented an increase but transient of IL-15 and IL-7 cytokines levels one week after chemotherapy. Furthermore, a high expansion of FoxP3$^+$regulatory T cells and PD-1$^+$ T cells was observed in all patients, related to IL-2 administration. Our results demonstrated that combining allogeneic NK cells transfer via intra-hepatic artery, cetuximab and a high-dose IL-2 is feasible, well tolerated and may result in clinical responses.

P.B1.09.15

Antigen presentation to CD169$^+$macrophages: translation to the human situation and coparison to DC-SIGN targeting

M. López-Venegas[1], *A. Barbaria*[1], *K. Olesek*[1], *L. Hoogterp*[1], *M. Ambrosini*[1], *H. Kalay*[1], *G. Storm*[2], *E. Puchhammer-Stöckl*[3], *Y. van Kooyk*[1], *J. den Haan*[1];
[1]Amsterdam UMC, Amsterdam, Netherlands, [2]Utrecht University, Utrecht, Netherlands, [3]Medical University of Vienna, Vienna, Austria.

Dendritic Cell Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN) and CD169/Sialic acid binding immunoglobulin type lectin I (siglec-1), are lectin receptors expressed by macrophages in secondary lymphoid organs and are implicated in antigen uptake. Our aim is to compare the efficacy of these lectin receptors with regard to antigen uptake and cross-presentation by monocyte-derived DCs (moDCs).
Fluorescent DC-SIGN and CD169-specific antibodies or liposomes containing the DC-SIGN and CD169 specific ligands Lewis Y and monosialodihexosylganglioside (GM3) were used to target gp100 melanoma antigen to moDCs. Binding and uptake of our targeting strategies was investigated by flow cytometry, while imaging flow cytometry was employed to study antigen routing. The determination of gp100 cross-presentation was assessed by coculturing targeted moDCs with gp100 specific HLA-A201-restricted T cells and analyzing IFNγ production. Our preliminary data show that liposome and antibody targeting of gp100 to DCSIGN and CD169 lead to effective binding, but also suggests that DCSIGN and CD169 lectin receptors may have a differential capacity to endocytose and to stimulate cross-presentation of antigens. Our studies will help to determine which lectin receptor is the most efficient to target antigens to for the activation of anti-melanoma T cell responses.

P.B1.09.16

Robust GMP manufacturing process with IL-2, fibronectin and anti-CD16 antibodies generates highly active human NK cell batches for cancer immunotherapy

K. Bröker[1], *U. Schumacher*[2], *R. Pörtner*[3], *H. Hoffmeister*[4], *E. Sinelnikov*[4], *S. Lüth*[1], *W. Dammermann*[1];
[1]Brandenburg Medical School, Brandenburg a. d. Havel, Germany, [2]University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [3]Hamburg University of Technology, Hamburg, Germany, [4]Zellwerk GmbH – HiPer-Group, Oberkrämer, Germany.

Introduction: NK cells are innate immune cells crucial for killing of infected and malignant cells. They are able to fight circulating tumor cells thereby preventing metastases formation which account for approximately 90% of all cancer-deaths. Thus, NK cells became interesting candidates for cancer immunotherapy and *ex vivo* manipulation and expansion of highly potent NK cells for adoptive transfer in patients an aim of paramount importance.
Methods: Human, CD3$^+$ T cell-depleted PBMCs were expanded in a fibronectin and anti-CD16 antibody-coated bioreactor in presence of IL-2 following GMP guidelines. Cells were analyzed for NK cell purity, expression of different chemokine receptors, cell adhesion molecules, activating receptors and death ligands as well as IFNγ production using flow cytometry. Further, cytotoxicity towards different tumor cell lines was assessed via LDH assays and flow cytometry-based degranulation assays.
Results: Upon expansion NK cell purity reached 85 to 96%. The cells showed expression of the chemokine receptors CXCR3, CXCR4 and CCR7 and the cell adhesion molecules L-selectin, LFA-1 and VLA-4. Further, they expressed the activating receptors NKp30, NKp44, NKp46, NKG2D, DNAM-1 and CD16, the death ligands Fas and TRAIL and produced IFNγ. NK cells showed cytotoxicity towards the tumor cell lines K562, PaCa5061, GC5830 and SKOV3.
Conclusion: We describe a novel approach for *ex vivo* NK cell expansion generating a set of highly potent NK cells which represent promising candidates for cancer immunotherapy. The GMP manufacturing process allows the use of these cells in clinical trials, i.e. adoptive NK cell transfer in patients.

P.B1.09.17

TNFa blockade overcomes resistance to anti-PD-1 in experimental melanoma

A. Montfort[1], *F. Bertrand*[1], *E. Marcheteau*[1], *C. Imbert*[1], *J. Gilhodes*[2], *T. Filleron*[2], *P. Rochaix*[2], *N. Andrieu-Abadie*[1], *T. Levade*[1,3], *N. Meyer*[1,3,4], *C. Colacios*[1,3], *B. Ségui*[1,3];
[1]INSERM U1037, CRCT, Toulouse, France, [2]Institut Universitaire du Cancer (IUCT), Toulouse, France, [3]Université Toulouse III, Toulouse, France, [4]CHU Purpan, Toulouse, France.

Anti-PD1 therapy has significantly improved the care of melanoma patients. However, about 40 to 70% of them do not display optimal response to treatment and responders often relapse and/or experience mild to severe immune related adverse events (irAEs). While anti-Tumor Necrosis Factor α (TNF) antibodies were successfully used in the clinic to help control irAEs, their impact on the anti-cancer immune response remained unknown. Our pre-clinical studies demonstrated that blocking the TNF/TNFR1 pathway potentiated the CD8$^+$-dependent anti-melanoma immune response in mouse. Moreover, blocking the TNF/TNFR1 pathway synergized with anti-PD1 treatment to impair tumor growth in mouse. In this context, we found anti-TNF prevented the anti-PD1 dependent upregulation of TIM-3 on T cells as well as activation-induced cell death thus favoring CD8$^+$ T cell accumulation in tumors. These results lead our team to take part to a phase 1b clinical trial aiming at evaluating the safety and tolerance of combining immune checkpoint inhibitors (ICI) to anti-TNF by metastatic melanoma patients (TICIMEL: NCT03293784).

P.B1.09.18

A non-small cell lung cancer (NSCLC) mouse model for improved preclinical validation of immune therapies

M. Kruchem;
Institut for Translationale Immunology, Mainz, Germany.

Introduction: Lung cancer has an overall dismal prognosis and only when diagnosed early, surgery and ablative therapies may offer a cure. Checkpoint inhibitors are a first good alternative treatment option promoting objective response rates and in some cases complete tumour remission. The failure of established treatment strategies and the therapeutic benefit of immune therapies highlight the need for additional research with tractable models.
Methods: For preclinical testing of immune therapies, we have generated a mouse model containing a conditional gene switch that sets off oncogenic K-Ras12V and inactivates p53 in lung progenitor (BASC), AT2 and Clara cells. This SKP model also contains two conditional reporter genes (EYFP and lacZ) to trace transformed lung cancer cells. Finally, we established several lung cancer cell lines from primary SKP tumours.
Results: Tamoxifen (TAM) injection in SKP mice resulted in reproducible lung tumor progression and the co-expression of EYFP and lacZ reporter genes. The specific expression of reporter genes in malignant cells allowed to exactly quantify the therapeutic benefit of different therapies/immune therapies. Injection of tumor-derived cell lines demonstrated that these cells metastasize to the brain, the peritoneum and the liver.
Conclusion: With the autochthonous SKP model and its metastasized SKP cell line derivatives any experimental immune therapy can be tested in an adequate and reliable preclinical setting. Co-expression of the two reporter genes differentiates between transformed tumor cells and non-malignant tumor-constituting cells thus allows to study the effect of different therapies for malignant tumor cells and non-transformed bystander cells.

P.B2.01 Environmental regulation anti-tumor responses - Part 1

P.B2.01.01

Assessment of promoter hypermethylation in tissue and blood of non-small cell lung cancer patients and association with survival

A. Ali, *P. Sohal, K. Upadhyay, A. Mohan, K. Madan, K. Luthra, S. Kumar, W. Rafi, R. Guleria;*
All India Institute of Medical Sciences, New Delhi, India.

Background Gene silencing by aberrant promoter hypermethylation is common in lung cancer and is an initiating event in its development. **Aim** To compare promoter hypermethylation frequency in serum and tissue of lung cancer patients with disease controls. Cyclin-dependent kinase inhibitor 2A (p16), a tumor suppressor gene, plays an important role in cell cycle regulation. O6 methylguanine DNA methyltransferase (*MGMT*) gene encoded protein is essential for genome stability **Method** 95 newly diagnosed untreated advance stage lung cancer patients and 50 cancer free matched controls were studied. Bisulfite modification of tissue and serum DNA was done; modified DNA was used as a template for methylation specific PCR analysis.
Survival was assessed for one year. **Results** Of 95 patients, 82% were non-small cell lung cancer (34% squamous cell carcinoma, 34% non-small cell lung cancer and 14% adenocarcinoma) and 18% were small cell lung cancer. Biopsy revealed that tissue of 89% and 75% of lung cancer patients and 85% and 52% of controls had promoter hypermethylated for MGMT (p=0.35) and p16 **(p<0.001)** gene, respectively. In serum, 33% and 49% of lung cancer patients and 28% and 43% controls were positive for MGMT and p16 gene**.** No significant correlation was found between survival and clinicopathological parameters. **Conclusion** High methylation frequency of p16 gene in tissue biopsy may be linked with early stages of carcinogenesis. Appropriate follow-up is required for confirmation of this finding.

P.B2.01.02

Implication of matrix metalloproteinases 9/2 and nitric oxide in the development of breast cancer: correlation with clinicopathological parameters

M. Amri[1], *A. Kouchkar*[2], *N. Benzidane*[2], *C. Touil-Boukoffa*[1];
[1]*University USTHB, Faculty of Biological Sciences, Laboratory of Cellular and Molecular Biology, Algiers, Algeria,* [2]*Department of anatomic-pathology, Centre Pierre and Marie Curie, Algiers, Algeria.*

Introduction: Many studies have demonstrated that nitric oxide (NO) plays a significant role in the multistep processing of carcinogenesis in breast cancer patients. These steps involve many inflammatory mediators like matrix metalloproteinases, in particular MMP 9 and 2. Thus, the aim of the present study was to investigate the activity levels of MMP9/2 and NO in breast cancer patients. **Methods:** MMP activities were assessed by a zymographic analysis in the sera of 125 patients carrying breast tumors and 20 healthy subjects as well as 63 breast tumors. Moreover, NO activity was investigated in the same samples by an enzymatic method. The results obtained were then correlated with the clinicopathological parameters. Moreover, immuno-histochemical staining was performed to analyze the tissue expression of CD68 (marker of infiltrating macrophages), uNOS (universal NO synthases), and NFkB. **Results:** The activities of MMPs and NO increased significantly in breast cancer patients compared with control subjects. Moreover, these activities were higher in patients with malignant tumors than in those with benign tumors both in sera and biopsy. They also correlate with tumors' size, type, stage, metastasis, and tissue expression of uNOS, CD68 and NFkB. **Conclusion:** Our results showed an association between high activities of MMPs (particularly MMP9) and NO and the development of breast malignant tumors. Interestingly, the serum MMP and NO level reflect the tissue levels. These findings suggest that serum levels of these molecules may be useful marker in monitoring breast carcinoma patients.

P.B2.01.03

Notch and Aiolos transcription factors in B CLL

J. Skelin[1], *L. Milkovic*[1], *I. Feliciello*[2], *B. Jelic Puskaric*[3], *M. Matulic*[4], *I. Kardum-Skelin*[3], *D. Radic-Kristo*[5], ***M. Antica***[1];
[1]*Ruđjer Bošković Institute, Zagreb, Croatia,* [2]*Dipartimento di Medicina Clinica e Chirurgia, Universita' degli Studi di Napoli Federico II, Napoli, Italy,* [3]*Department of Clinical Cytology and Cytogenetics, Merkur Univesity Hospital, Zagreb, Croatia,* [4]*University of Zagreb, Faculty of Science, Zagreb, Croatia,* [5]*School of Medicine, University of Osijek, Zagreb, Croatia.*

B-cell chronic lymphocytic leukaemia (B-CLL) is characterized by increasing accumulation of monoclonal lymphocytes. The leukemic clone is characterised by simultaneous expression of the CD5 antigen together with specific B-cell antigens CD19, CD20 and CD23. We used this feature of the abnormal B-cell clone expressing $CD5^+CD19^+$ and characterised the individual cell groups within the sample at the transcriptional and protein level in order to study the aberrant developmental stages of the B cells in this disease regarding the roles of Ikaros transcription factor Aiolos, the Notch signalling pathway and its target genes Hes and Deltex. We also established a coculture system with the Delta-like ligand transfected OP9 cells which is mandatory for T cell development in order to explore the activation potential of the Notch receptors we found expressed also by B-CLL cells. Although well explored in T-cell acute lymphoblastic leukaemia, the Notch specific pathway involvement in B-CLL has been contradictory so far. In addition to Notch, we and others have previously shown that a member of the Ikaros family of zinc -finger proteins, Aiolos has a very high expression in B-CLL lymphocytes and there is evidence supporting its role in the survival of other leukaemic B cells. We analysed these genes simultaneously and tested apoptosis regulation by Notch activation on co-cultures of the malignant clone on the OP9DLL1 cell line.

P.B2.01.04

Relation between dendritic cells and regulatory T cells in mammary neoplasm in dogs

P. H. L. Bertolo*, M. C. Rosolem, M. B. Conceição, P. R. Moreira, R. O. Vasconcelos;*
Sao Paulo State University (UNESP), Jaboticabal, Sao Paulo, Brazil.

Introduction - The malignant mammary tumors have several ways of evading the immune system, including the modulation of dendritic cells (DCs), by interfering with their maturation, resulting in inefficient presentation of antigens to T cells and consequent induction of immunological tolerance. Therefore, this study aimed to evaluate the relationship between DCs and regulatory T cells (Treg) in the simple type canine mammary carcinomas, using the immunohistochemical technique. Methods - Ten samples of mammary gland without tumor (G1) and 40 samples of mammary neoplasms (G2: adenomas, G3: papillary carcinomas, G4: tubular carcinomas and G5: solid carcinomas) were submitted to immunodetection of Treg cells (FOXP3+), CD4 and CD8 T cells, MHC-II, myeloid DCs (immature and mature), cytokines TGF-β, IL10, Indoleamine 2,3-dioxygenase (IDO) and chemokine receptors (CCR6 and CCR7). Results - Most of the cells, cytokines and immunological receptors showed positive correlation within the tumor population and controls evaluated, mainly on the fixed effect "age" that had high positive correlation with the tumor size and with CCR6. It was observed that there was a relationship between immature / mature myeloid DCs and Treg cells, as well as TGFβ and IL-10, and the IDO enzyme showed a marked presence in the malignant samples. Conclusion - Immunodetection of CCR6 and CCR7 occurred mainly in the most aggressive tumors, where CCR6 had a high relation with older patients and with larger tumors, which, in the end, may reflect that the aging of the immune system may be more of a pro- tumor.

P.B2.01.05

Cytotoxic and helper T- and innate lymphoid cell subsets in myelodysplastic syndromes

M. Boy[1,2], *A. Celia*[1,2], *J. Villemonteix*[1,2], *G. Henry*[1], *L. Amable*[1], *L. Comba*[1], *J. Klibi*[1], *A. Lim*[1], *H. Yssel*[3], *A. Caignard*[1], *K. Benlagha*[1], *J. Di Santo*[4], *P. Fenaux*[1,2], *L. Adès*[1,2], *A. Toubert*[1,2], *N. Dulphy*[1,2];
[1]*UMRS-1160 INSERM, Hôpital Saint-Louis, Centre Hayem, Paris, France,* [2]*Université Paris Diderot, Sorbonne Paris Cité, Hôpital St. Louis, Paris, France,* [3]*U1135 INSERM, Hôpital Pitié-Salpêtrière, Département d'Immunologie, Paris, France,* [4]*U1223 INSERM, Institut Pasteur, Département d'Immunologie,, Paris, France.*

Introduction: Myelodysplastic syndrome (MDS) is a group of heterogeneous clonal disorders arising in the bone marrow (BM). MDS evolution is dominated by the risk of progression into acute myeloid leukemia. In addition, 10 to 30% of MDS patients present autoimmune disorders (AID).
We hypothesized that MDS could lead to perturbations in cytotoxic and cytokine-producing lymphocyte subsets, including Natural Killer (NK) cell, Innate Lymphoid Cells (ILCs) and T cells. These functional deficiencies could be associated with inefficient immunosurveillance and AID.
Materials and Methods: Expressions of activation markers, chemokine receptors, and regulatory molecules were determined by flow-cytometry in order to evaluate phenotypic and functional perturbations of T-cells, NK-cells and ILCs in peripheral blood samples from MDS patients, as compared to healthy controls, using conventional and non-supervised analytical methods. Quantitative PCR was used to investigate the expression of lymphocyte subset-specific transcription factors (T-bet, Eomes, Gata-3, RORγt), and functional markers (perforin, IFN-γ).
Results: A reduction in the percentage of circulating ILC subsets was observed in patients, as compared to healthy controls. The NK-cell phenotype showed alterations in the expression of activating and inhibitory receptors. Moreover, perturbations in the distribution of the various T lymphocyte subsets and in the expression of immune checkpoint molecules were observed.
Conclusions: Altogether, these results are in favor of a profound perturbation in the frequency of peripheral blood lymphocyte subsets in MDS patients. The data warrant to further analyze the impact of the BM microenvironment in MDS on the lymphoid phenotype, as well as to determine its association with AID.

P.B2.01.06

Shared CCR7-CCL19 pathway in NK and melanoma cells marks the clinical progression and suggests a new immune escape strategy in melanoma patients

C. M. Cristiani[1], *V. Ventura*[1], *A. Turdo*[2], *T. Apuzzo*[2], *M. Capone*[3], *G. Madonna*[3], *D. Mallardo*[3], *C. Garofalo*[1], *E. Selinger*[1], *E. Simeone*[3], *A. M. Grimaldi*[3], *C. Coracò*[4], *E. Staaf*[5], *R. Tallerico*[1], *G. del Zotto*[6], *E. Gulletta*[1], *A. Moretta*[7], *K. Kärre*[5], *M. Todaro*[2], *P. A. Ascierto*[3], *E. Carbone*[1];
[1]*University "Magna Graecia" of Catanzaro, Catanzaro, Italy,* [2]*University of Palermo, Palermo, Italy,* [3]*Istituto Nazionale Tumori Fondazione "G. Pascale", Naples, Italy,* [4]*Istituto Nazionale Tumori Fondazione, Naples, Italy,* [5]*Karolinska Institutet, Stockholm, Sweden,* [6]*Istituto Giannina Gaslini, Genoa, Italy,* [7]*University of Genoa, Genoa, Italy.*

Introduction. Changes within the immune response that can be associated to and can mark melanoma clinical progression are poorly investigated. Here, we identified selective changes in NK cells repertoire and serum cytokine pathological perturbation characterizing the transition from stage III to stage IV. The potential role of CCR7-CCL19 axis in metastatic process was dissected as well.
Materials and Methods. PBMCs from healthy donors and melanoma patients were characterized for their immune profile and activation status. Their sera as well as melanoma cells supernatants were characterized for cytokines/chemokines content. Melanoma primary cells and cancer stem cells (CSCs) were analyzed for CCR7 and NK immunomodulatory ligands expression, as well as for their susceptibility to NK cells cytotoxicity.
Results. The frequency of $CCR7^+CD56^{bright}$ NK cells reached its peak in stage IV melanoma patients, together with the serum levels of CCL19. CCL19 serum concentration may derive from the ectopic production by melanoma cells, since it was detectable in melanoma cells supernatants but not in those from other tumors cells. A small cell subpopulation within primary melanoma cells selectively co-expressed CCR7, PD-L1 and Galectin-9 and was characterized by low levels of MHC class I molecules. This phenotype was shared by melanoma CSCs, which were highly susceptible to NK cells-mediated killing.
Conclusions. The accumulation of tumor produced CCL19 in the blood may promote the selective blood metastatic spread of the $CCR7^+$PD-$L1^+$Gal-9^+HLA-class I^{low} melanoma CSCs and subvert the tumor immune response by preventing $CD56^{bright}$ NK cells migration in sentinel lymph nodes.

P.B2.01.07

The influence of short-term and long-term ibrutinib treatment on the HLA-DR expression on leukemic and T cells in chronic lymphocytic leukemia

G. Gabcova[1], *Z. Mikulkova*[1], *G. Manukyan*[2], *T. Papajik*[3], *P. Turcsanyi*[3], *R. Fillerova*[1], *T. Dyskova*[1], *V. Smotkova Kraiczova*[1], *S. Zehnalova*[4], *P. Gajdos*[4], *R. Urbanova*[3], *M. Kudelka*[4], *E. Kriegova*[1];
[1]*Department of Immunology Faculty of Medicine and Dentistry Palacky University, Olomouc, Czech Republic,* [2]*Laboratory of Molecular and Cellular Immunology, Institute of Molecular Biology NAS RA, Yerevan, Armenia,* [3]*Department of Hemato-Oncology, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc, Czech Republic,* [4]*Department of Computer Science, Faculty of Electrical Engineering and Computer Science, VSB-Technical University of Ostrava, Ostrava, Czech Republic.*

There is the first evidence about the changes in the kinetics of B cell antigen receptor (BCR) internalization of neoplastic cells in chronic lymphocytic leukemia (CLL) after the short-term and long-term administration of ibrutinib. We aimed to assess the influence of short-term and long-term ibrutinib treatment on the HLA-DR expression on CLL and T cells.
The immunophenotyping of CLL and immune cells in peripheral blood was performed in 16 high-risk CLL patients treated with ibrutinib. Early after ibrutinib administration, the HLA-DR expression on CLL cells reduced (P=0.019), accompanied by an increase in CLL cell counts in peripheral blood (P=0.001). *In vivo* reduction in the HLA-DR expression was confirmed by *in vitro* culturing of CLL cells with ibrutinib at protein and mRNA levels (P<0.01). The decrease in HLA-DR on CLL cells after the first month was followed by the gradual increase of its expression by the 12th month (P=0.016).
The one-month follow-up resulted in elevated absolute counts of CD4+ (P=0.002) and CD8+ (P<0.001) cells as well as CD4+ and CD8+ cells bearing HLA-DR (P<0.01).
The long-term administration of ibrutinib was associated with increased numbers of CD4+ bearing HLA-DR (P=0.047), along with a trend to higher CD4+ and CD8+ absolute cell counts in the majority of patients. Our results provide the first evidence of the time-dependent immunomodulatory effect of ibrutinib on CLL and T cells. The clinical consequences of time-dependent changes in HLA-DR expression in ibrutinib treated patients deserve further investigation. **Grant support:** MZ ČR VES16-32339A, IGA UP_2018_016, MH CZ-DRO (FNOL, 00098892)

P.B2.01.08

Using multi-parameter analysis to dissect the systemic immune landscape of breast cancer patients

H. Garner[1,2], *M. Brüggemann*[1,2], *K. Vrijland*[1,2], *C. Hau*[1,2], *M. Kok*[1], *K. E. De Visser*[1,2];
[1]*Netherlands Cancer Institute, Amsterdam, Netherlands,* [2]*Oncode Institute, Amsterdam, Netherlands.*

Metastatic breast cancer remains the principal cause of death for breast cancer patients. Whilst strategies for treating the primary tumour have markedly improved, our ability to prevent or treat metastasis remains considerably less effective. It is now widely accepted that the tumour microenvironment plays a critical role in determining tumour biology. However, tumour-induced perturbations to the host reach far beyond the tumour microenvironment, evoking systemic changes that remain relatively underexplored.
In this project, we set out to extensively characterise the systemic immune landscape of breast cancer patients of different molecular subtypes and at differing disease stages compared to age- and sex-matched healthy controls. By employing multi-parameter flow cytometry analysis in conjunction with unbiased clustering and dimension reductionality techniques we are able to characterise the systemic immune landscape at a single cell level.
Preliminary findings show that advanced, metastatic triple negative breast cancer (TNBC) patients have considerably expanded neutrophil frequency and these neutrophils appear more homogeneous compared to healthy controls. Furthermore, TNBC patients have considerably reduced frequency of circulating lymphocytes yet these lymphocytes show an increased propensity to produce IL-17 and increased expression of the immune checkpoint receptor PD1. This analysis technique will allow us to generate a systemic immune "fingerprint" of different breast cancer molecular subtypes and different disease stages.

P.B2.01.09

Ig-like transcript 2 (ILT2) suppresses T cell function in chronic lymphocytic leukemia

S. Gonzalez, *S. Lorenzo-Herrero, C. Sordo-Bahamonde, A. López-Soto, A. González-Rodríguez, Á. R. Payer, M. Villa-Álvarez;*
Universidad de Oviedo. IUOPA, Oviedo, Spain.

Introduction: Chronic lymphocytic leukemia (CLL) is a heterogeneous and incurable disease characterized by malignant B cell accumulation and a profound immune dysregulation. T-cell dysfunction is caused by signaling mediated by inhibitory receptors such as PD-1 and CTLA-4. In this study, we describe the role of a novel inhibitory receptor, Ig-like transcript 2 (ILT2), in the pathogenesis of CLL by mediating T-cell exhaustion.
Materials and Methods: Surface expression of ILT2 and its ligands was analyzed in different lymphocyte subsets obtained from 52 patients with CLL and 20 healthy donors by flow cytometry. The role of ILT2 in T-cell function (activation, proliferation and cytokine production) was determined *in vitro* by using an ILT2-blocking antibody.
Results: In CLL, ILT2 expression was markedly decreased in leukemic cells and upregulated in CD4 and CD8 T lymphocytes, especially in those patients with bad prognostic features, such as chromosome 11q deletion. Moreover, the expression of ILT2 ligand was profoundly dysregulated on leukemic cells. ILT2 expression was associated with the suppression of the effector function of T cells and ILT2 blockade restored their activation and proliferation without affecting B cells. Further, ILT2 blockade increased IL-2 production and downregulated the production of cytokines that promote the survival of leukemic cells, such as IFN-γ.
Conclusions: Expression of ILT2 and its ligands is deeply dysregulated in patients with CLL. Our data suggest that ILT2 blockade may restore T cell activation, proliferation and cytokine production, suggesting that ILT2 may be a new potential therapeutic target for the treatment of patients with CLL.

P.B2.01.10

Changes in rat microglia metabolic profile after treatment with doxorubicin and its complex with C_{60} fullerene *in vitro*

Y. Hurmach, *S. Prylutska, M. Rudyk, K. Stepura, Y. Maiboroda, O. Shuliak, D. Hladun, S. Stepanenko, V. Svyatetska, Y. Prylutskyy, L. Skivka;*
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine.

Microglia are resident CNS macrophage-like cells involved in neuroimmunity. Also they are the predominant immune cell infiltrating brain neoplasms, such as glioblastoma. Preclinical studies suggest that doxorubicin (Dox) had a strong antineoplastic activity against gliomas. Dox conjugated to nanoparticles demonsrtates enhanced anti-cancer activity through the mechanisms including tumor-associated microglia targeting. The aim of the work was to evaluate the effect of Dox in complex with C_{60} fullerene on metabolic profile of rat microglia *in vitro*. Microglial cells from adult Wistar rats were treated with C_{60} fullerene, Dox, and C_{60} + Dox complex for 30 min. Metabolic profile of microglial cells was characterized by the arginine metabolism as well as by the phagocytosis and reactive oxygen species (ROS) generation. Treatment of microglial cells with with C_{60} + Dox complex resulted in increased reactive nitrogen species generation. Meanwhile, phagocyte arginase activity remained unchanged after the treatment. It indicates pro-inflammatory shift of microglia arginine metabolism. ROS production by microglial cells treated with C_{60} + Dox complex was 2 times higher than that in untreated cells and 1.5 times higher than that after treatment with Dox used alone. Phagocytosis of microglial cells treated with Dox was 2.5 times higher than that in untreated cells and 3 times higher than that after treatment with C_{60} + Dox complex. Complexation of Dox with C_{60} fullerene potentiates its modulatory effect on microglia metabolic profile.

P.B2.01.11

Sall4 oncogene is an immunogenic antigen presented in various HLA-DR contexts.

m. kroemer[1,2], *L. Spehner*[1], *M. Ben khelil*[1], *P. Letondal-Mercier*[1], *L. Boullerot*[1], *Y. Godet*[1], *O. Adotévi*[1,2], *C. Borg*[1,2];
[1]*EFS/BFC/UMR1098, Besançon, France,* [2]*CHRU Besançon, Besançon, France.*

Purpose: To investigate the immunoprevalence of SALL4-derived peptides in healthy volunteers and cancer patients. Experimental Design: A multistep approach including prediction algorithms was used to design *in silico* SALL4-derived peptides theoretically able to bind on common HLA-DR and HLA-A/B molecules. The presence of T-cell responses after a long term T-cell assay (28 days) against SALL4 was monitored in 14 healthy donors and the presence of T-cell responses after a short term T-cell assay (10 days) was monitored in 67 cancer patients using IFN-γ ELISPOT assay. A T-cell clone specific for the immunoprevalent A18K-derived peptide was isolated, characterized and used as a tool to characterize the natural processing of A18K. Results: A SALL4 specific T-cell repertoire was present in healthy donors (8/14) and cancer patients (29/67) after short term T-cell assay. We further identified two immunoprevalant SALL4-derived peptides, R18A and A18K, which bind MHC-class II. In parallel, an A18K specific Th1 clone recognized monocyte derived Dendritic Cell (moDC) loaded with SALL4 containing cell lysate. The level of IFN-γ secreted by specific T-cell clone was greater in presence of moDC loaded with SALL4 containing cell lysate (49.23 ± 14.02%) than with moDC alone (18.03 ± 3.072%) (p= 0.0477)
Conclusion: These results show for the first time immunogenicity of SALL4 oncogenic protein-derived peptides, especially A18K and R18A peptides and make them potential targets for personalized medicine. Thus, SALL4 possess major characteristics of a tumor antigen.

P.B2.01.12

Modulation of pulmonary microbiota by antibiotic or probiotic aerosol therapy: a new strategy to promote immunosurveillance against lung metastases

V. Le Noci[1], *S. Guglielmetti*[2], *S. Arioli*[2], *C. Camisaschi*[3], *M. Sommariva*[4], *C. Storti*[1], *A. Balsari*[1,4], *E. Tagliabue*[1], *L. Sfondrini*[4];
[1]*Molecular Targeting Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy,* [2]*Dipartimento di Scienze degli Alimenti, Nutrizione e Ambiente (DeFENS), Università degli Studi di Milano, Milan, Italy,* [3]*Immunotherapy Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy,* [4]*Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Milan, Italy.*

Immunological tolerance in the lung microenvironment is essential to control inflammation in response to inhaled particulates, but it also creates a permissive milieu for the setting of lung metastasis. Since the lung microbiota is implicated in pulmonary tolerance, we explored whether its manipulation via antibiotics or probiotics aerosolization in C57BL/6 mice limits melanoma metastasis by subverting local immune suppression and boosting immune responses. Here, we show that in lungs of vancomycin/neomycin aerosolized mice a decrease in bacterial load was associated to a reduction in regulatory T cells (Tregs). Moreover, the enhanced activation of lung T and NK cells paralleled the significant reduction of lung metastases in mice aerosolized with antibiotics and intravenously injected with melanoma B16 cells. Reduction of lung metastases also occurred in lung transplanted with bacteria isolated from the bronchoalveolar lavage of antibiotic-treated mice (*Morganella morganii, Campylobacter* sp); likewise, transplantation of bacteria isolated from untreated mice (*Paenibacillus glucanolyticus, Bacillus clausii)* attenuated the anti-metastatic effects of antibiotics. Aerosolized probiotic *Lactobacillus rhamnosus*, a human commensal bacterium, strongly limited B16 metastases implantation and promoted immune activation as well. Furthermore, probiotics or antibiotics improved the therapeutic effects of dacarbazine, a chemotherapeutic agent used in metastatic melanoma patients, in advanced B16 metastases-bearing mice. Our results reveal for the first time that the balance of immunostimulatory and immunosuppressive commensal bacteria in lungs is relevant in creating an immunological permissive milieu for metastatization. Thus, targeting lung microbiota via probiotic or antibiotic aerosolization represent a new therapy to prevent metastases and enhance the response to chemotherapy.

P.B2.01.13

IgG subclass switching and clonal expansion in human colorectal cancer

R. Liu*, Q. Zhang, H. Liu;*
Institute of Gastroenterology and the Sixth Affiliated Hospital, Guangzhou, China.

B lymphocytes play an important role in the maintenance of intestinal homeostasis. However, the subsets and roles of B lymphocytes in human colorectal cancer are not clear. Here, we found that B cells showed a lymphatic-like distribution in the steady state but a disorganized distribution pattern in human colorectal cancer tissue. These results led us to suspect that the change in distribution pattern may affect B cell differentiation and development. Our further study showed that IgM^+ and IgA^+ B cell subsets skewed to IgG^+ B cell subsets in tumor microenviroment. Consistent with this, tumor-infiltrating B cells featured shorter complementarity-determining (CDR3) regions compared with B cells isolated from normal tissues. More importantly, we also detected higher IgG4 levels in the plasma of colorectal cancer patients compared with healthy donors , suggesting that B cell subsets change in tissues can be reflected in the patient's body fluids. Evidence for antibody class switching and antibody maturation in human colorectal cancer, support the involvement of B cells in human colorectal cancer immunity.

P.B2.01.15

Serum chemokine profiling reveals candidate biomarkers for recurrence and immune infiltration in ovarian cancer

A. Mlynska[1], *G. Salciuniene*[1], *K. Zilionyte*[1], *B. Intaite*[1], *A. Barakauskiene*[2], *V. Pasukoniene*[1];
[1]*National Cancer Institute, Vilnius, Lithuania,* [2]*Vilnius University, Vilnius, Lithuania.*

The management of advanced ovarian cancer is challenging due to the high frequency of recurrence, often associated with the development of resistance to platinum-based chemotherapy. Molecular analyses revealed the complexity of ovarian cancer with particular emphasis on the immune system that may contribute to disease progression and response to treatment. Chemokines orchestrate the cross-talk between cancer and immune cells, and therefore present as potential biomarkers, reflecting the tumor microenvironment. We examined a panel of circulating CC and CXC chemokines in the serum of 40 high-grade ovarian cancer patients prior to primary surgery. We also analyzed the level of immune infiltration in tumors.The preoperative levels of chemokines differ between patients. Elevated levels of circulating CXCL4+CCL20+CXCL1 combination can discriminate patients with shorter recurrence-free and overall survival. Serum CCL17 has a potential to select platinum-resistant tumors. In half of the patients, we detected the presence of intratumoral lymphocytes, as well as high expression of T cell response-associated genes. Circulating CXCL9+CXCL10 combination can distinguish immune-infiltrated tumors that are more likely to recur. Our results suggest that profiling of circulating chemokines in ovarian cancer patients may provide valuable information regarding tumor chemosensitivity and immune infiltration. We show that combinations have better prognostic utility than single chemokines and may serve as patient stratification tools.

P.B2.01.16

Diverse functions of CR3 (CD11b/CD18) and CR4 (CD11c/CD18) β_2-integrins expressed by human B lymphocytes

Z. Nagy-Baló[1], *S. Lukácsi*[1,2], *B. Mácsik-Valent*[1,2], *Z. Bajtay*[1,2], *A. Erdei*[1,2];
[1]*Department of Immunology, Eötvös Loránd University, Budapest, Hungary,* [2]*MTA-ELTE Immunology Research Group, Eötvös Loránd University, Budapest, Hungary.*

CR3 and CR4 are known for long to participate in adhesion and migration of myeloid cells. The expression and function of these β_2-integrins on human B lymphocytes however, has not been extensively studied yet.
Investigating the CD11b and CD11c expressing human B cell line BJAB we found that blocking CR4 with a CD11c specific antibody, a significant, up to 50 % reduction of adhesion could be observed on fibrinogen coated surface, in contrast to the effect of the anti-CD11b antibody. Therefore CR4 dominates adhesion of B cells over CR3, showing a division of labour of these two β_2-integrins, similarly to that observed previously in the case of myeloid cells (Sándor et al., PloSOne, 2016). Employing cells of the Daudi line we found that blocking of either receptor decreases the migration towards SDF-1α by 30-40 %. Our current experiments include also B cells isolated from tonsils, which express CR4 upon stimulation by PMA.
Further, we analysed the function of these two receptors on B cells of chronic lymphocytic leukaemia (CLL) patients expressing varying levels of CR3 and CR4, since adhesion and migration are important for survival of these malignant cells. Our ongoing experiments show that these receptors participate in both functions also on CLL B cells at different scales depending on the level of expression. We also found that inhibition of adhesion by blocking with PLL-PEG lowers the rate of proliferation of CLL B cells triggered by different stimuli.

P.B2.01.17

Exploring the effects of tetraspanin CD37-deficiency on metabolic signaling during B-cell lymphomagenesis

R. Peeters[1], *A. Hoekstra*[2], *R. Stienstra*[3], *C. Berkers*[2], *E. Jansen*[1], *A. Van Spriel*[1];
[1]*Department of Tumor Immunology, RIMLS, Radboudumc, Nijmegen, Netherlands,* [2]*Department of Biochemistry and Cell Biology, University of Utrecht, Utrecht, Netherlands,* [3]*Department of Internal Medicince, Radboudumc, Nijmegen, Netherlands.*

Introduction: Immune cells employ a metabolic state that fits their specific needs. These needs fluctuate throughout their lifespan. Metabolic alterations are induced by intracellular and extracellular signals that are coordinated by membrane receptors. Abnormal composition of these receptors can have drastic effects on cell faith. The tetraspanin superfamily of 4-transmembrane proteins controls membrane protein organization. Absence of the immune-specific tetraspanin CD37, results in spontaneous B-cell lymphoma development in mice. Importantly, diffuse large B-cell lymphoma (DLBCL) patients lacking CD37 on tumor cells have a significantly worse prognosis. CD37 controls the activity of Akt kinase which plays a central role in metabolic regulation and cell survival. We therefore set out to establish the effects of CD37-deficiency on the metabolic faith of healthy B-cells and its potential role in lymphomagenesis.
Objective: Provide more insight into the metabolic pathways underlying CD37 function in healthy B cells and during lymphomagenesis, potentially finding modes of clinical intervention.
Methods: Metabolite abundance in WT and $CD37^{-/-}$ of human B-cell lines and primary murine B-cells was assessed with mass spectrometry (MS). The metabolic analyzer Seahorse XF96 was used to directly measure oxidative phosphorylation (OXPHOS) and glycolysis activity in live cells. Furthermore, mitochondrial phenotyping of B-cells was carried out using confocal microscopy.
Results: Preliminary results indicate that B-cells without CD37 display lower metabolic activity. WT cells contain higher absolute levels of OXPHOS-, and glycolysis-associated metabolites. Furthermore, WT B-cells portray higher oxygen consumption and extracellular acidification, indicating more active OXHPHOS and glycolysis respectively.

P.B2.01.18

B cells in esophago-gastric adenocarcinoma are highly differentiated, organize in tertiary lymphoid structures and produce tumor-specific antibodies

H. A. Schlößer[1], *M. Thelen*[2], *A. Lechner*[3], *K. Wennhold*[2], *B. Gathof*[4], *R. Gilles*[4], *E. Cukuroglu*[5], *J. Göke*[6], *A. Quaas*[4], *C. Bruns*[4], *A. H. Hölscher*[4], *M. S. von Bergwelt-Baildon*[7,8,2];
[1]University of Cologne and Center for Molecular Medicine Cologne, Cologne, Germany, [2]Center for Molecular Medicine Cologne, Cologne, Germany, [3]Ludwigs Maximilian University Munich, Munich, Germany, [4]University of Cologne, Cologne, Germany, [5]Genome Institute of Singapore, Singapore, Singapore, [6]Genome Insitute of Singapore, Singapore, Singapore, [7]Ludwig Maximilian University Munich, Munich, Germany, [8]German Cancer Consortium (DKTK), Heidelberg, Germany.

*Introduction*Tumor-infiltrating lymphocytes (TILs) are correlated to prognosis of several kinds of cancer. Most studies focused on T cells, while the role of tumor-associated B cells (TABs) has only recently gained more attention. TABs contain subpopulations with distinct functions, potentially promoting or inhibiting immune responses. This study provides a detailed analysis of TABs in gastro-esophageal adenocarcinoma (EAC).*Methods*Single cell suspensions of tumor samples (n=54), mucosa (n=43), lymph nodes (n=42) and peripheral blood mononuclear cells (PBMC, n=88) of EAC and PBMC of healthy controls (n=20) were studied by flow cytometry.
A panel of 34 tumor-associated antigens (TAAs) expressed in EAC was identified based on public databases and TCGA data to analyze tumor-specific B cell responses using a LUMINEX™bead assay and flow cytometry. Spatial distribution of TABs was analyzed by confocal immunofluorescence-microscopy.ResultsB cells were elevated in primary tumor samples compared to PBMCs of gastric cancer patients or normal mucosa. Subset-analyses of TILs revealed increased proportions of differentiated and activated B cells and enrichment for follicular T helper cells. TABs were organized in tertiary lymphoid structures (TLS) at the invasive tumor margin. Structural analyses of TLS and the detection of tumor-specific antibodies against one or more TAAs in 48.1% of analyzed serum samples underline presence of anti-tumor B cell responses in EAC. B cells were decreased in tumors with expression of Programmed Death Ligand 1 or impaired HLA-I expression.ConclusionsAnti-tumor B cell responses are an additional and underestimated aspect of EAC. These results are of immediate translational relevance to emerging immunotherapies.

P.B2.01.19

Evaluation of serum iron in tumorigenesis and malignancy of ovarian cancer

N. TOUNSI[1], *A. Benyelles-Boufennara*[2], *B. Djerdjouri*[3];
[1]University of sciences and technology Houari Boumedienne, Alger, Algeria, [2]Department of Department of Pathological anatomy, Public Health Center Pierre and Marie Curie, Mustapha Bacha Hospital (Algiers, Algeria)., Algiers, Algeria, [3]University of sciences and technology Houari Boumedienne, Algiers, Algeria.

Introduction: Ovarian cancer is a lethal malignancy that recorded in 2012, 238719 new cases and caused 151917 deaths, worldwide. It represents the fifth most common women cancer in Algeria, with a worse prognosis for more than 66% of new cases 821 deaths in 2012.
The goal of our study was to evaluate the contribution of serum iron in Algerian ovarian cancer patient's progression.
Materials and Methods: Hematoxylin and eosin (H&E)-stained sections of ovarian tumors were examined for diagnosis (benign or malignant tumor) and staging. Sera from 40 ovarian tumor patients before treatment and 30 controls were sampled and used for seric iron and CA-125 evaluation.
Results: H&E stained sections of ovarian cancer showed that among the 40 patients, 30 are malignant tumor and 10 benign tumor. The 30 malignant tumor displayed 2 patients in stage 1, 12 patients in stage 2, 11 patients in stage 3 and 5 patients in stage 4.
Iron levels decreased by 17% ($p < 0.05$) while CA-125 increased by 98% ($p < 0.001$) in malignant ovarian cancer patients, compared to healthy individuals. NO significant correlation were shown between seric iron and CA-125 levels (r= 0.2879, $p > 0.05$). Despite the altered iron levels, it was not significantly associated to tumoral stage progression (r= 0.1265, $p > 0.05$).
Conclusion: Our preliminary data suggests that seric iron cannot be taken as supportive biomarkers for the diagnosis of ovarian cancer but may partially emphasizes the iron microenvironment enrichment for ovarian cancer initiation.

P.B2.01.20

SERUM AND URINARY LEVELS OF CD222 IN CANCER DISEASES: ORIGIN AND DIAGNOSTIC VALUE

K. Vičíková[1], *E. Petrovčíková*[1], *P. Maňka*[2], *J. Drach*[3], *H. Stockinger*[4], *V. Leksa*[1];
[1]Institute of Molecular Biology, Bratislava, Slovakia, [2]BSP Sizar s.r.o, Bratislava, Slovakia, [3]University Hospital Vienna, Department of Medicine I, Vienna, Austria, [4]Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectology and Immunology, Medical University in Vienna, Vienna, Austria.

The mannose 6-phosphate/insulin-like growth factor 2 receptor (CD222, M6P/IGF2R) is a multifunctional transmembrane type I receptor, mostly localized intracellularly, less on the surface of all types of mammalian cells. It is known both to transport lysosomal enzymes through their mannose 6-phosphate moieties and to internalize extracellular ligands like insulin-like growth factor 2 or plasminogen. CD222 is involved in regulation of cell proliferation, migration, T cell activation, and apoptosis. Soluble CD222 has been found in higher concentrations in sera of liver disease patients. In this study, we analysed the level of CD222 present in body fluids, namely in serum and urine, of cancer patients. We found significantly elevated levels of soluble CD222 in sera of cancer patients compared to healthy controls irrespective of the type of disease. The urine CD222 levels were increased specifically in breast cancer and multiple myeloma. In contrast to serum, CD222 was present within CD222-positive exosomes in urine pointing to different origins of CD222 present in various human body fluids. Based on this work, we propose serum soluble CD222 as a general biomarker for tumorigenesis.

P.B2.01.21

Study of the biological effects of lactoferrin on the prostate cancer cells with varying sensitivity to hormonal therapy

T. Zadvornyi, *N. Lukianova, V. Chekhun;*
RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Kyiv, Ukraine.

Introduction. Correction of prostate cancer (PCa) hormone resistance is one of the top research directions of biology exploration of this localization. One of the perspective approaches is the search of low-toxic substances that increase treatment efficacy.
Aim: to investigate biological effects of lactoferrin (LF) and to find out the possibility of its use to reduce the malignancy degree of human PCa cells by modifying their phenotype.
Materials and methods: The hormone-sensitive (LNCaP) and hormone-independent (DU-145) human PCa cell lines were cultured with an exogenous LF. The expression levels of ER, PR, Her2/neu, Ki-67, E- and N-cadherin, were monitored by immunohistochemical analysis. The levels of miRNAs were assessed, using q-PCR. The invasive activity of the cells was examined using a standard invasive test according to the manufacturer's instructions.
Results. We established that cultivation of human PCa cell lines with exogenous LF resulted in lowering of steroid hormone receptor expression (ERa and PR). The decrease in the expression of the Ki-67 under the influence of exogenous LF was observed in both cell lines. Also, we established the decrease of invasive activity - by 40% and 30% in DU-145 and LNCaP cell lines, respectively. We found that under the action of exogenous LF there was an increase in the level of expression of oncogenic and oncosuppressive miRNAs in both cell lines.
Conclusions: Thus, we have shown that under the influence of exogenous LF there are changes in phenotypic characteristics and levels of oncogenic and oncosuppressive miRNAs.

P.B2.02 Environmental regulation anti-tumor responses - Part 2

P.B2.02.01

Evaluation of local immune response after silencing of IL-10 or IL-10R expression in MC38 tumors

N. Anger, *A. Szczygieł, K. Węgierek, J. Mierzejewska, M. Napierała, E. Pajtasz-Piasecka, J. Rossowska;*
L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland.

A growing tumor is composed of a variety of cells and factors, which collectively form the tumor microenvironment (TME). One of the cytokines, which is frequently upregulated in cancer is interleukin 10. The majority of reports indicate that IL-10 is a suppressive cytokine with a pro-tumoral effect. However, IL-10 can also enhance the anti-tumor response. The aim of our research was to evaluate the role of IL-10 in MC38 murine colon carcinoma microenvironment through silencing of IL-10 or IL-10R expression.
Mice with subcutaneously growing MC38 tumors were intratumorally inoculated with lentivectors silencing IL-10 or IL-10R expression (shIL-10 LVs or shIL-10R LVs). Characterization of the tumor microenvironment was performed on the 4th and 6th day after LV inoculation. Percentage of tumor infiltrating subpopulations of myeloid and lymphoid cells and their activation stage were evaluated by flow cytometry.
A simultaneous accumulation of CD8$^+$ T cells and PMN-MDSCs was observed in TME on the 4th and 6th days after inoculation with shIL-10 LVs. However, shIL-10R LVs and control LVs induced an antiviral response characterized by high influx of CD8$^+$ T cells into tumor on the 6th day after inoculation.
The gathered data indicates that inoculation with LVs induced a strong antiviral response. This effect seemed to be diminished by reduction of IL-10 in TME. Additionally, the early influx of CD8$^+$ T cells observed on the 4th day after inoculation suggests a CTL-dependent antitumor response.
This work was financed by National Science Centre, Poland (grant no 2014/15/N/NZ4/04817).

P.B2.02.02
Modeling cancer immunomodulation using epithelial organoid cultures

Y. E. Bar-Ephraim[1,2], *K. Kretzschmar*[1,2], *P. Asra*[1,2], *Z. Sebestyen*[3], *J. Kuball*[3], *H. Clevers*[1,2,4];
[1]*Hubrecht Institute, Utrecht, Netherlands,* [2]*Oncode Institute, Utrecht, Netherlands,* [3]*UMC, Utrecht, Netherlands,* [4]*Princess Maxima Center, Utrecht, Netherlands.*

Colorectal carcinoma (CRC) is one of the most prevalent forms of cancer which develops in a multi-step process from lesions in healthy colon tissue. While mutations in cancerous epithelial cells drive the process of tumorigenesis, interaction of the tumor with the immune system and subsequent evasion from immune-mediated destruction is essential for tumor progression.
Epithelial organoids provide a platform which allows culturing of cancerous and healthy epithelium while retaining tissue-of-origin identity over a prolonged culture period. As such, epithelial organoids are a reliable system to model many biological processes, ranging from normal epithelial differentiation to tumor development. Also, tumor-derived organoids have shown potential to be used in patient-specific drug screens, making a critical step towards personalized medicine.
Here, we report a new method to study immune-cancer interactions and assess modulation of the immune response by CRC. We derived organoids from CRC samples and show by transcriptional profiling that organoids maintain differential expression of immune modulatory molecules present in primary tumors. Further, we have set up a co-culture system for organoids and T cells to assess immunoreactivity. Indeed, upon co-culture with TCR transgenic $CD8^+$ T cells, organoid killing and cytokine production by T cells was only observed when co-cultured organoids were pulsed with TCR specific native peptides but not with control peptides. In conclusion, our method presented here allows for investigation of immune cell-tumor interaction in vitro and how immunomodulators can be utilized to stimulate tumor eradication. Implementation of this system may thus lead towards new avenues of patient-specific treatment.

P.B2.02.03
$CD68^+$ cells not implicated in Ncf1-mediated tumor progression

M. Y. Bonner*, R. Holmdahl;*
MIR, Karolinska Institutet, Solna, Sweden.

Cancer is the second leading cause of death globally, according the WHO, with the number of deaths from cancer expected to increase by 70% over the next two decades. The purpose of this study is to advance our understanding in cancer biology and anti-cancer immune response in order to facilitate the development of improved anti-cancer therapies needed to address this concern. Levels of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide are known to increase as premalignant cells evolve into malignant lesions. ROS is also know to play a role in immune response and communication. The aim of our study is identify which immune cell subtype is able to inhibit tumor growth through NOX2-ROS inhibition. A mutation in the Ncf1, component of the NOX2 complex prevents ROS production in B10.Q Ncf1*/* mice. Recent results point to impaired tumor growth in mice with the Ncf1 mutation, in agreement with published studies. Interestingly, when the B10.Q Ncf1*/* mice acquire a functional Ncf1 gene only in $CD68^+$ macrophages, B10.Q Ncf1*/* MN, they also present similarly impaired tumor growth in relation to the wildtype B10.Q mice. We then tested to the potential of $CD11c^+$ cells mediated tumor progression through functional and non-functional Ncf1 molecules. Our results indicate that the potential of $CD11c^+$ lineage involvement in Ncf1 mediated tumor progression in B16F10 melanoma and LLC Lewis lung carcinoma tumor models.

P.B2.02.04
Assessing the role of myeloid cell GCN2 in anti-tumor immune responses

F. Cichon[1], *J. K. Sonner*[1], *K. Deumelandt*[1], *L. Wolf*[1], *E. Green*[1], *W. Wick*[1,2], *M. Platten*[1,3];
[1]*German Cancer Research Center, Heidelberg, Germany,* [2]*Department of Neurology and National Center of Tumor Diseases, Heidelberg, Germany,* [3]*Department of Neurology, University Medical Center Mannheim, Mannheim, Germany.*

Nutrient deprivation is a hallmark of the tumor microenvironment and exerts significant suppressive influence. The tryptophan catabolism has been identified as a central pathway restricting T cell immunity in tumors. In clinical trials inhibitors of the rate-limiting enzyme that mediates tryptophan depletion, indoleamine-2,3-dioxygenase (IDO), have been tested as monotherapy and in combination with checkpoint inhibition without positive results thus far. Based on the identification of the stress kinase general control non-derepressible 2 (GCN2) as a central hub for sensing tryptophan deprivation and mediating downstream effects of the immunoregulatory enzyme IDO, further investigations are required to define the role of immune cell GCN2 in the tumor microenvironment to understand the mechanism of IDO-mediated immunosuppression. We demonstrate that the deletion of GCN2 within the immune compartment promotes resistance to T cell activating immunotherapy (glycoprotein 100 peptide vaccine and immune checkpoint inhibition targeting programmed death-ligand 1) in experimental B16 melanoma in full knock-out mice and bone marrow chimera. Since we have recently reported that T cell GCN2 is dispensable for suppression of B 16 melanoma rejection, we are focussed on the role of myeloid cell GCN2 for anti-tumor immune responses. *In vitro* genetic ablation of *Gcn2* reinforced immunosuppressive capacities of bone-marrow derived macrophages. In addition, *Gcn2* deletion in bone marrow-derived dendritic cells suppressed cytotoxic responses of co-cultured gp100-specific T cells. In conclusion, our results suggest that GCN2 mediated downstream signaling exerts substantial influence on both, efficient antigen presentation and production of immunosuppressive factors and thereby significantly modulates anti-melanoma immune responses during checkpoint Blockade.

P.B2.02.05
CombImmunotherapy: poly(I:C) primes glioblastoma for PD-L1 blockade via lymphocyte attraction and activation

J. De Waele[1], *E. Marcq*[1], *J. Van Audenaerde*[1], *J. Van Loenhout*[1], *C. Deben*[1], *K. Zwaenepoel*[2], *E. Van de Kelft*[3], *D. Van der Planken*[3], *T. Menovsky*[2], *J. Van den Bergh*[1], *Y. Willemen*[1], *P. Pauwels*[1,2], *Z. Berneman*[1,2], *F. Lardon*[1], *M. Peeters*[1,2], *A. Wouters*[1], *E. Smits*[1];
[1]*University of Antwerp, Antwerp, Belgium,* [2]*Antwerp University Hospital, Edegem, Belgium,* [3]*AZ Nikolaas, Sint-Niklaas, Belgium.*

Novel therapies are needed to address to abysmal prognosis of glioblastoma patients. Immunotherapy requires combination strategies to unlock its full potential. Here, we investigated the immunomodulatory capacities of poly(I:C) on glioblastoma cells and its combinatorial potential with programmed death ligand (PD-L) blockade.
Primary human glioblastoma cells were cultured from residual tumour tissue obtained from standard surgery of glioblastoma patients. Phenotyping was performed using flow cytometry (FCM), immunohistochemistry, qRT-PCR and multiplex electrochemiluminescence. Toll-like receptor 3 (TLR3) signalling was inhibited using chloroquine. Lymphocyte migration was studied using a transwell FCM assay, and activation using FCM and ELISA. Additional PD-L1/PD-L2 blockade was evaluated using ELISA and CSFE-labelled T-cell proliferation.
Poly(I:C) stimulated a pro-inflammatory secretome by gliobolastoma cells, including type I interferons (IFN), interleukin-15, reduced transforming growth factor β, and chemokines CXCL9, CXCL10, CCL4 and CCL5. Concomitantly, PD-L1 and PD-L2 expression on glioblastoma cells was stimulated via TLR3 signalling. Poly(I:C)-treated glioblastoma cells doubled $CD8^+$ T-cell attraction, and to a lesser extent $CD4^+$ T cells, in part via ligands for CXCR3 and CCR5, while natural killer cell migration was not affected. Lymphocytes cocultured with poly(I:C)-treated glioblastoma cells showed enhanced activation (CD69, IFN-γ) and cytotoxic potential (CD107a, granzyme B). Additional blockade of PD-L1, but not PD-L2, further propagated this immune activation.
Our results show that poly(I:C) triggers glioblastoma cells to secrete cytokines which attract and activate $CD8^+$ T cells, following which blocking of the elevated tumoural PD-L1 further reinforces immune activation. In conclusion, our data proposes poly(I:C) to strengthen PD-L1 blockade in glioblastoma.

P.B2.02.06
CDK5 involvement in the tumor microenvironment

F. Farina[1,2], *M. Quintavalle*[2], *M. Locati*[1];
[1]*Università degli Studi di Milano, Milano, Italy,* [2]*Istituto Clinico Humanitas, Rozzano, Italy.*

Cyclin-dependent kinase 5 (CDK5) is a serine/threonine kinase belonging to the CDK family. Several works highlighted CDK5 role in cancer progression and invasiveness both in solid tumors and in hematopoietic malignancies. Until now, no previous work described CDK5 role in Tumor Associated Macrophages. To study CDK5's involvement in macrophages polarization, we performed both gain- and loss-of-function studies. Preliminary *in vitro* experiments were performed on THP1 monocytic cell line, differentiated into M0 macrophages. Once differentiated, macrophages polarization was induced after IFNγ (M1) and IL-4 or IL-10 (M2) treatment. Lentiviral overexpression of p35 in M0 macrophages was employed as a gain-of-function model, while lentiviral overexpression of CDK5-specific shRNA was employed as a loss-of-function model. We observed that CDK5 is highly expressed in macrophages at basal level and it is downregulated after inflammatory stimuli. We measured a reduction in CDK5 protein expression after M1 phenotype induction (IFNγ), indicating that CDK5 is necessary for a correct M1 polarization. Conversely, p35 overexpressing macrophages showed a decreased pro-inflammatory gene expression. Therefore, these data suggested a clear CDK5 role in TAMs via a p35/CDK5 activation pathway and possible CDK5 involvement in tumor progression. CDK5 silencing, induced a reduction in podosome formation. These data clarify results obtained in vitro and suggest a possible correlation between p35/CDK5 deregulation and cancer invasion. In addition, CDK5 expression in TAMs might be used as prognostic marker for the outcome evaluation of breast cancer patients. We aim to demonstrate that CDK5 inhibition might be a viable anti-metastatic strategy to treat invasive breast cancer.

P.B2.02.07

CD16^{+}NKG2A^{high} NK cells from Tumor draining lymph nodes correlate with stage in Breast Cancer patients

***A. Frazao**, M. Messaoudene, N. Nunez, E. Piaggio, N. Dulphy, A. Toubert, A. Caignard;*
INSERM, Paris, France.

We characterized the Natural killer (NK) cells that infiltrate tumor draining (TD) lymph nodes (LN), the first site of metastasis of breast cancers (BC). We analyzed by flow cytometry the phenotype of NK cells from TD-LN, (including non-invaded (NI) and metastatic (M)-LN from BC patients) and also NK cells from healthy donor (HD)-LN. First, we show that NK cells from paired NI and M-LN display similar phenotype and M-LN contained low percentages of tumor cells that express ULBP2 and HLA class I molecules. Compared to HD-LN, TD-LN NK cells highly express NCR, NKG2D and NKG2A receptors and characterized by elevated CD62L and CXCR3 expression. TD-LN contained a major compartment of activated CD56^{+}CD16^{+} NKG2A^{high} and these NK cells are prominent in Stage IIIA BC patients. We found that a subset of LN-NK cells express PD-1. TD-LN NK cells degranulate efficiently after co-culture with BC cell lines. Cytokine activated TD-LN NK cells exerted higher lysis of BC cell lines than HD-LN NK cells and preferentially lysed the HLA class-I^{low} PD-L1^{low} MCF-7 BC cell line. The expression of inhibitory receptor NKG2A and checkpoint PD-1 by TD-LN NK cells from BC indicate their potential as targets for immunotherapies using anti-NKG2A and/or anti-PD1 mAbs.

P.B2.02.08

Slan+ monocytes and NK cells contribute to a tumor microenvironment that induces a p21-dependent growth arrest in melanoma cells

***F. Funck**[1,2], J. Pahl[2], A. Cerwenka[2], K. Schäkel[1];*
[1]Dermatology, University Hospital Heidelberg, Heidelberg, Germany, [2]Medical Faculty Mannheim, University Heidelberg, Mannheim, Germany.

The cell types of the immune system orchestrate effective anti-tumor responses and often eliminate malignant cells before primary tumors or metastasis can arise. In the mouse system, it was recently reported that patrolling monocytes initially detect newly formed metastasis and reduce metastatic load by recruiting NK cells. slan^{+} monocytes (slanMo) represent a subset of human CD14^{dim}CD16^{+} monocytes (homolog of patrolling monocytes in mice) and were previously identified in melanoma metastasis. slanMo are capable of initiating anti-tumor responses based on the secretion of proinflammatory cytokines and the interaction with NK cells is highlighted by an IL-12/IFN-γ dependent positive feedback loop that results in high levels of TNF-α and IFN-γ. Here, we address the question whether the cytokine milieu generated by co-culturing these two cell types influences the growth of melanoma cells and can lead to senescence induction. To this end, we incubated melanoma cell lines with supernatants from slanMo/NK co-cultures. Supernatant treatment resulted in a severely reduced proliferation rate, increased Senescence-associated beta-Galactosidase staining, and a senescence phenotype characterized by strong p21 (CDKN1A) upregulation. This phenotype could be abolished by combined TNF-α and IFN-γ neutralization. We provided evidence that NK cells migrate towards activated slan supernatants *in vitro*. In addition, we validated the presence of slanMo in melanoma metastasis prior and after immunotherapy, together supporting a mechanism similar to patrolling monocytes in the mouse system. Our data suggests that slanMo are present in melanoma metastasis and contribute to a pro-inflammatory immune microenvironment that inhibits the growth of melanoma cells. Funded by RTG2099.

P.B2.02.09

Ni(II) complexes with Mannich bases affect viability and proliferation of rat tumor and non-tumor (bone marrow, macrophages, lymphocytes) cells

***M. Glavcheva**[1], T. Zhivkova[1], B. Andonova-Lilova[1], L. Dyakova[2], R. Tudose[3], E. Mosoarca[3], O. Costisor[3], R. Alexandrova[1];*
[1]1Institute of Experimental Morphology, Pathology and Anthropology with Museum, Sofia, Bulgaria, [2]2Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria, [3]Institute of Chemistry Timisoara of the Romanian Academy, Timisoara, Romania.

In recent years there has been an increasing interest in the antitumor and immunomodulating activities of metals and metal compounds. The aim of the present study was to evaluate the influence of four Ni(II) complexes with ligands containing the antipyrine moiety N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) or N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN) on viability and proliferation of cultured rat tumor and non-tumor cells. The following cell cultures were used as model systems in our investigations: permanent cell line (LSR-SF-SR) established from transplantable sarcoma in rat induced by Rous sarcoma virus strain Schmidt-Ruppin; primary cultures from tumor growths developed after a s.c. implantation of LSR-SF-SR cells (7.5 x 10^6 cells/animal) in inbred Wistar rats (PRSC) as well as from bone-marrow cells (BMC), peritoneal macrophages (PM) and spleen lymphocytes (SL) of the same tumor bearing animals and healthy rats. The investigations were performed by MTT test, trypan blue dye exclusion technique, double staining with acridine orange and propidium iodide and colony-forming method. The compounds were applied at concentrations of 10, 50, 100 and 200 μg/ml for 24h, 48h and 72h. The results obtained revealed that: i) $Ni_2(BAMP)(CH_3COO)_4$ and $Ni_2(BAMP)(Cl)_4$ are more pronounced cytotoxic agents as compared to $Ni(TAMEN)(ClO_4)_2$ and $Ni(TAMEN)(NCS)_2$ for both tumor and non-tumor cells; ii) BMC are relatively more sensitive to the toxic effects of Ni(II) complexes as compared to the other used cell culture models; iii) Both ligands (BAMP, TAMEN) do not significantly decrease viability and proliferation of the treated tumor and non-tumor cells. The authors gratefully acknowledge the EU Grant BG05M2OP001-2.009-0019-C01 from 02.06.2017.

P.B2.02.10

TGF beta compromises STING-induced IFN alpha/beta production and tumor regression in spontaneous tumors

***M. V. Guérin**[1], F. Regnier[2], J. M. Weiss[3], V. Feuillet[2], L. Vimeux[2], M. Thoreau[2], G. Renault[2], E. Donnadieu[2], A. Trautmann[2], N. Bercovici[2];*
[1]Cochin Institute INSERM U1016 CNRS UMR8104, Univ Paris Descartes, Paris, France, [2]Cochin Institute INSERM U1016 CNRS UMR8104, Univ Paris Descartes, Paris, France, [3]Univ Medical Center Freiburg, Freiburg, Germany.

Background: The rate of tumor growth and the responsiveness to therapies depend not only on intrinsic properties of malignant cells but also on the tumor microenvironment. Here, we examined in which conditions targeting the ubiquitous cytosolic protein STING in spontaneous tumors can trigger the production of type I IFN in the tumor microenvironment, inducing tumor regression. Materials and methods: Mice with spontaneous mammary tumors (MMTV-PyMT) received a single intraperitoneal injection of the STING ligand DMXAA. We examined the evolution of tumor growth and performed a molecular analysis of the tumor and immune infiltrate by flow cytometry, fluorescence imaging and transcriptomics. Results: We show that IFNα/β release that conditioned a swift recruitment of neutrophils, followed by a rise in CD8 T cells and monocytes in transplanted tumors, was impaired at an early stage in tumor cells and immune cells infiltrating spontaneous tumors. One reason is that TGFβ, abundant in such tumors, prevents the phosphorylation of IRF3 following STING stimulation. Blocking TGFβ restored the production of IFNα and facilitated tumor regression in the mammary tumor model. Conclusions: These data highlight that IFNα/β production is crucial to initiate an efficient anti-tumor immune response. We provide evidence for a determinant role of TGFβ in IFNα/β deficient induction in spontaneous tumors. This calls for further development of combined strategies allowing STING-dependent tumor rejection to take place. This work was granted by the Ligue Contre le Cancer Ile de France.

P.B2.02.11

TGFB1 polymorphisms may identify gastric adenocarcinoma patients with high risk of metastasis and lower survival rate

***I. Juarez**[1], A. Gutierrez[2], A. Blazquez[2], E. Ovejero[2], I. Lasa[2], A. Lopez[2], R. Gomez[2], J. M. Martin-Villa[1];*
[1]Universidad Complutense de Madrid, Dpt. of Immunology, Madrid, Spain, [2]Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Madrid, Spain.

Transforming growth factor β 1 (TGF-β1) is a cytokine involved in the development and malignancy of tumours. Several works attribute a dual effect to the cytokine in the cancer evolution depending on its levels and the stage of the disease. TGFB1 gene presents several single nucleotide polymorphisms (SNP), related with TGF-β1 levels.
We analysed four SNPs (rs1800468, rs1800469, rs1800470, rs1800471) in a group of 78 patients with gastric adenocarcinoma to assess the association between the TGFB1 and tumor progression.
Patients were classified as type I, II, III (non-metastatic) or IV (metastatic), according to their TNM stage. Upon DNA isolation, the polymorphisms were genotyped. PBMC were isolated and stimulated with PMA-Ionomicin and TGF-β1 was measured by ELISA. Survival curves analysis was also performed.
rs1800468-G/A genotype was present in 30% of metastatic patients compared to 10.3% of non-metastatic patients (p=0.049, OR=3.17). rs1800469-T/T was absent in metastatic patients, and present in the 19.1% of non-metastatic patients (p=0.03). The combined haplotyped ACTG was present in 15% of the metastatic patients as compared to 3.2% of non-metastatic (p=0.019, OR=7.65). rs1800469-C/C polymorphism yielded a lower expression of TGF-β1 than the C/T or T/T (1.44 and 1.41-fold respectively) variants. Likewise, the rs1800470 T/T polymorphism produced lower TGF-β1 amounts than C/C variant (0.73-fold). Finally, PBMC of rs1800469T/T-rs1800470C/C bearing patients produce higher TGF-β1 upon stimulation (1.4-fold, p=0.025) and have better survival-rates (85.5%) than rs1800469C/C-rs1800470T/T patients (28.1%)
These polymorphisms may us enable to pinpoint metastasis-prone patients, who would need more aggressive therapeutic approaches upon diagnosis.

P.B2.02.12

Impact of photodynamic therapy on the regulation of human immune system in the context of hepatocellular carcinoma

A. Kumar[1], *O. Morales*[1], *B. Leroux*[1,2], *C. Frochot*[3], *S. Mordon*[4], *N. Delhem*[1], *E. Boleslawski*[1];
[1]*Lille Biology Institute, Lille, France,* [2]*INSERM Unit U1189-ONCO THAI, Lille, France,* [3]*LRGP, UMR-CNRS 7274, University of Lorraine, Nancy, France,* [4]*INSERM, U1189-ONCO THAI, Lille, France.*

Introduction: With development in Photodynamic Therapy (PDT), scientists are trying to test the therapy in different cancer models. However, the immunological impact of the therapy is largely unknown. As immune-escape is the major hallmark for proliferating cancers, hence, we aim to evaluate the impact of 5-Aminolevulinic acid (5-ALA) mediated PDT on HCC cell lines: HuH7 (p53 over expression), HepG2 (wild type p53) and Hep3B (partially deleted p53).
Methodology: The expression of Delta-Aminolevulinic Acid Dehydratase (ALAD) and Protoporphyrinogen Oxidase (PPOX) was analyzed through qPCR. The optimal 5-ALA and illumination dosage was determined by treating cells with varying 5-ALA concentration and illumination duration, followed by cellular mitochondrial metabolism analysis. Thereafter, the cells were treated with these PDT parameters, the 'conditioned medium' was recuperated and used to culture cancer cells to analyze the cellular viability and proliferation.
Results: qPCR proves that HCC cell lines express ALAD and PPOX enzymes. Subsequent treatment of the cell lines reveal cancer cell death along with minimal change in proliferation when cultured with PDT treated conditioned media.
Conclusion: The anti-cancer therapy caused cancer cell death with a cell death pattern corresponding to the state of p53, along with an inhibition of cancer cell proliferation suggesting towards cell-cycle checkpoint regulation. Our preliminary studies have also showed that 5-ALA PDT can induce an immune-regulatory microenvironment, while its capability to prompt an immunogenic cell death and the role of cancer secreted exosomes remains to be studied.

P.B2.02.13

Predictive and prognostic value of circulating and tumor-associated NK cells in HER2-positive breast cancer patients treated with neoadjuvant therapy

A. Muntasell[1], *S. Servitja*[2], *F. Rojo*[3], *M. Cabo*[1], *S. Santana*[1], *I. Tusquets*[2], *B. Bermejo*[4], *M. Martinez*[4], *O. Arpi*[1], *M. Martínez-García*[2], *M. Costa-García*[5], *P. Eroles*[4], *I. Vázquez*[6], *L. Serrano*[6], *C. Vilches*[7], *A. Rovira*[2], *A. Lluch*[4], *J. Albanell*[2,5], *M. López-Botet*[1,5];
[1]*Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain,* [2]*Hospital del Mar-CIBERONC, Barcelona, Spain,* [3]*IIS 'Fundación Jiménez Diaz', Madrid, Spain,* [4]*Institute of Health Research INCLIVA, Valencia, Spain,* [5]*University Pompeu Fabra, Barcelona, Spain,* [6]*Hospital del Mar, Barcelona, Spain,* [7]*Instituto de Investigación Sanitaria Puerta de Hierro, Madrid, Spain.*

We investigated the value of distinct NK cell-related variables for predicting pathological complete response (pCR) in primary breast cancer patients undergoing anti-HER2 antibodies (mAbs)-based neoadjuvant treatment.
The immunophenotype of baseline circulating NK cells and the CD16A 158V/F genotype were analysed by multiparametric flow cytometry and PCR in a prospective cohort of patients recruited between 2014 and 2016 (n=64). Tumor-infiltrating NK cell numbers were assessed by double immunohistochemistry (CD56+CD 3-) in diagnostic tumor biopsies from recruited from 2008 to 2016 (n=139). NK cell-related variables were correlated with pCR adjusted for prognostic factors.
CD16A 158V/F genotype was not associated with pCR. Baseline circulating CD57+ NK cells and tumor-infiltrating NK cell numbers respectively showed an inverse and a positive association with pCR (p=0.029 and p<0.0001), independent of clinicopathological factors. Levels of <64% circulating CD57+NK cells and of ≥3 tumor-infiltrating NK cells 50 high-power fields (HPF) predicted pCR [OR 5.52 (95% CI, 1.68-18.058) and OR 44.6 (95% CI, 14.3-138,7), respectively]. In addition, circulating CD57+ and tumor-infiltrating NK cell numbers associated with longer disease-free survival. Of note, circulating CD57+NK cells inversely correlated with tumor-infiltrating NK cell numbers, according to the reduced frequency of CD57+NK cells in tumor-associated immune infiltrates.
Circulating CD57+ and tumor-infiltrating NK cells predict the likelihood of achieving pCR to anti-HER2 mAb-based neoadjuvant treatment. Our data point to the putative importance of tumor-infiltrating NK cells and the fitness of the circulating NK cell repertoire for the efficacy of anti-HER2+ breast cancer neoadjuvant therapies.
F.S: Worldwide Cancer Research Foundation; AECC Foundation ; PIE-ISCIII; CIBERONC

P.B2.02.14

The ecto-ATPDase CD39 is involved in the acquisition of the immunoregulatory phenotype by M-CSF-macrophages and ovarian cancer tumor-associated macrophages: Regulatory role of IL-27

L. Papargyris[1], *S. d'Almeida*[1], *G. Kauffenstein*[2], *C. Roy*[2], *L. Basset*[1], *P. Jeannin*[3], *M. Grégoire*[1], *Y. Delneste*[1], *J. Tabiasco*[1];
[1]*INSERM U1232, ANGERS, France,* [2]*INSERM U1083, ANGERS, France,* [3]*INSERM U1232, Laboratoire d'Immunologie et d'Allergologie, CHU Angers, ANGERS, France.*

Objectives: Tumor-associated macrophages (TAM) are immunosuppressive cells that can massively accumulate in the tumor microenvironment (ME). In patients with ovarian cancer (OC), their density is associated with poor prognosis. Targeting mediators that control the generation/differentiation of immunoregulatory macrophages (Mϕ) may represent therapeutic challenge to overcome tumor-associated immunosuppression.
Methods: Our laboratory has previously shown that (i) in vitro monocytes treated with M-CSF or GM-CSF induce the generation of M2 and M1 Mϕ, respectively and (ii) that in vitro differentiated M-CSF-Mϕ are similar to TAM. We analyzed the expression of the membrane ectonucleotidase CD39 in Mϕ subsets and its role in the biology of M-CSF-Mϕ and ovarian cancer TAM.
Results: We observed that $CD14^+$ $CD163^+$ TAM isolated from ovarian cancer patients and that in vitro generated M-CSF-Mϕ express high levels of CD39 compared to M1-type GM-CSF-Mϕ. CD39 hydrolyzes ATP into extracellular adenosine that exhibits potent immunosuppressive properties when signaling through the A2A adenosine receptor. CD39 blockade diminished some of the immunosuppressive functions of $CD163^+$ $CD39^{high}$, such as IL-10 production. We identified the cytokine IL-27, secreted by tumor-infiltrating neutrophils, located close to infiltrating $CD163^+$ Mϕ, as a major rheostat of CD39 expression and, consequently, of the immunoregulatory properties of Mϕ. Accordingly, blocking IL-27 down regulated CD39 and PD-L1 expression and IL-10 secretion by M-CSF-Mϕ.
Conclusions: These data suggest that targeting molecules that maintain the immunosuppressive phenotype of TAM (IL-27-induced CD39, CD115 ligands) could give substantial benefit to the treatment of ovarian cancer.

P.B2.02.15

Carboplatin-cucurbituril complex: antitumour activity with reduced immunotoxicity

N. Knauer[1], ***E. Pashkina***[1], *E. Kovalenko*[2], *A. Aktanova*[3], *A. Ermakov*[3], *V. Kozlov*[1];
[1]*Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation,* [2]*Nikolaev Institute of Inorganic Chemistry, Novosibirsk, Russian Federation,* [3]*Novosibirsk State Medical University, Novosibirsk, Russian Federation.*

Cancer is the leading cause of mortality worldwide. Platinum(II)-based cytostatic drugs are actively used for antitumour therapy. However they have a plenty of side effects such as immunosupression because of their toxicity. This problem can be potentially solved by using nanocarriers for drug delivery which allow to reduce systemic toxicity without loss of efficacy. Cucurbiturils, macrocyclic cavitands, are promising tools for this purpose.
Herein, we study the effect of carboplatin, cucurbit[7]uril and carboplatin-cucurbit[7]uril complex (1:1) on B16 melanoma cells and on the primary culture of peripheral blood mononuclear cells (PBMCs) of healthy volunteers. The cells were cultivated in RPMI 1640 media with 10% of fetal calf serum in presence of drugs under study in concentration 0.01-0.3 mM for 48 h (B16) and 72 h (PBMCs). Cytotoxic effect was evaluated by MTT test, proliferative activity was evaluated using CFSE labeling.
It was found that complex in concentration 0.3 mM exhibits higher toxicity towards tumor cells than carboplatin in the same concentration. At the same time, the complex and carboplatin in concentration 0.3 mM have the equal toxicity towards PBMCs. Furthermore, recently, we have demonstrated that cucurbit[7]uril in concentration 1 mM has proper toxicity towards either tumor cells or PBMCs. Cucurbit[7]uril can also suppress aCD3-induced PBMCs proliferation.
Our findings suggest that cucurbit[7]uril can be a prospective nanocarrier for decreasing the toxicity of cytostatic drugs which also has its own immunomodulative effects.
The work was supported by RFBR grant No. 18-315-00158.

P.B2.02.16

A lipid mediated paracrine signaling network stimulates tumor associated macrophage development in cancer and metastasis

V. Rai, *R. Ray;*
Institute of Life Sciences, Bhubaneswar-751023 India, Bhubaneswar, India.

Tumor microenvironment consist of dynamic interactions between tumor cells and the surrounding non-transformed cells. Inflammatory cells constitute a major population of the non-transformed cells. Tumor associated macrophages (TAMs), the predominant population of inflammatory cells have major roles in cancer progression and metastasis but the exact stimulus and triggers for the tumor cells-macrophage interaction remains unclear. Autotaxin or lysophospholipaseD (LysoPLD) catalyses the synthesis of lyophosphatidic acid - the smallest phospholipid from lysophosphatidylcholine by its enzymatic action. Autotaxin is implicated in breast cancer, ovarian cancer and many other cancers. Lysophosphatidic acid (LPA) is involved in numerous biological processes encompassing cell growth, cell proliferation, cell migration, cancer and metastasis. LPA effects are mediated on different cell types via its cognate G-protein coupled receptors (GPCRs) or non-receptor pathways.

Our recent study has shown that LPA converts monocytes into macrophages both in mice and humans and has an important role to play with immune cells. Here, we show that cancer cells in tumor growth are associated with tumor associated macrophages via a signature paracrine link. Our studies identify previously unknown signaling link between tumor cells and macrophages. Furthermore, we identify that suppression of this paracrine network can suppress tumor growth. This study suggests that inhibition of this paracrine network may act as a new therapeutic approach to control cancer and metastasis.

P.B2.02.17

Interest of NK cells to counteract resistance to target therapies in melanoma

***L. Rethacker**, A. Frazao, M. Avril, A. Caignard;*
INSERM, Paris, France.

Melanoma incidence is increasing for several decades and metastatic melanoma patients still have a poor prognosis. Since the identification of activating mutations in B-RAF in 50% of melanoma patients treatment with BRAF (vemurafenib) and then BRAF+MEK (cobimetinib) inhibitors is the first line treatment for patients bearing a tumor with a BRAF mutation. Despite high response rates of the development of resistance and relapse after a few months is frequent. To find the best combined treatment, we have investigated how resistance to these inhibitors interferes with melanoma cell immunogenicity to Natural Killer (NK) cells. From 3 BRAF mutated melanoma cell lines, we have generated vemurafenib resistant variants (R). Paired sensible (S) and R cells to vemurafenib displayed similar mutational profile, comparable cell growth kinetics and the growth of R variants is maintained in presence of vemurafenib. We found that the resistance to BRAF is associated to increased immunogenicity to NK cells. First, NK cell activation (degranulation and IFNγ production) is strongly increased in response to R cells. The lysis of R cells by NK cells was significantly increased. Compared to S cell lines, R variants displayed increased expression of NKG2D ligands (MICA, ULBP2), increased Fas, and TRAILR expression. The acquisition of resistance is associated to increased NK immunogenicity, increased TRAIL induced apoptosis. These findings outline the interest of combined target therapies and NK based immunotherapy for melanoma patients.

P.B2.02.18

Utilization of high-frequency irreversible electroporation (H-FIRE) to modulate the tumor microenvironment and promote systemic immune system activation in breast cancer

***V. M. Ringel-Scaia**[1,2], S. L. Coutermarsh-Ott[2], R. M. Brock[1,2], K. E. Huie[2], N. Beitel White[3], M. F. Lorenzo[3], R. V. Davalos[3], I. C. Allen[1,2,4];*
[1]Graduate Program in Translational Biology, Medicine, and Health, Virginia Tech, Blacksburg, United States, [2]Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Blacksburg, United States, [3]School of Biomedical Engineering and Sciences, Virginia Tech-Wake Forest University, Blacksburg, United States, [4]Department of Biomedical Science, Virginia Tech Carilion School of Medicine, Roanoke, United States.

Introduction: Breast cancer is among the most common malignancies in the US; 1 in 8 women will develop invasive breast cancer in her lifetime. Despite promising treatments for breast cancer, there is no cure once metastatic disease has developed. Thus, new therapeutics to address metastases are direly needed. High-frequency irreversible electroporation (H-FIRE) is a particularly novel and emerging therapeutic approach for tumor ablation. This technique utilizes a series of high-frequency bipolar electric pulses applied via electrodes inserted directly into the tumor to induce cancer cell death. Our overarching hypothesis predicts local treatment of the breast tumor with H-FIRE will stimulate both the innate and adaptive immune system, leading to systemic anticancer response and improved survival.
Materials and Methods: We utilized a mouse 4T1 mammary tumor model and applied H-FIRE to the primary tumor. We evaluated changes in the size of the primary tumor after treatment, as well as metastatic burden and gene expression in the primary tumor at the conclusion of the model.
Results: Here, we show H-FIRE treatment of the primary tumor results in near complete ablation and a shift in the tumor microenvironment from immunosuppressive to pro-inflammatory. Local H-FIRE treatment also significantly reduces 4T1 metastases in animals with an intact immune system, indicating increased engagement of a systemic anti-tumor immune response and improved activation of the adaptive immune system.
Conclusions: We anticipate this novel tumor ablation technology will improve conventional treatment strategies and complement emerging immunotherapy approaches targeting primary tumors and metastatic lesions.

P.B2.02.19

Serum pre-inflammatory cytokines TNF-a and IL-6 have higher predictive strength compared to metalloproteases and markers of tumor activity, bone metabolism and cell apoptosis in breast cancer patients with bone metastases.

*A. Notopoulos[1], **A. Sarantopoulos**[2], P. Notopoulos[3], K. Psarras[4], C. Likartsis[1], E. Alevroudis[1], I. Petrou[1], Z. Iconomou[1], G. Meristoudis[1], E. Zaromytidou[1], A. Doumas[5];*
[1]Department of Nuclear Medicine, Hippokration General Hospital of Thessaloniki, Thessaloniki, Greece, [2]2nd Department of Internal Medicine, Aristotle University of Thessaloniki, Hippokration GHTH, Thessaloniki, Greece, [3]Neuroinformatics Laboratory, Technological Institute of Central Macedonia, Thessaloniki, Greece, [4]2nd Propedeutical Surgery Department, Aristotle University of Thessaloniki, Hippokration GHTH, Thessaloniki, Greece, [5]2nd Department of Nuclear Medicine, Aristotle University of Thessaloniki, AHEPA University GHTH, Thessaloniki, Greece.

Objective: To evaluate the predictive strength of 32 serum markers in breast cancer patients with bone metastases (BC+BM) under treatment.**Methods**: The serum level of all markers has been determined in 153 BC+BM patients (a) at their enrollment in the study, (b) one month later, and (c) after six months. We created a conventional "scan score" based on the number, size and metabolic activity of BM in the initial bone scintigraphy. Levels of p53, bcl-2, TRAIL, caspase-3, FasL, Fas, MMP-1, MMP-2, TIMP-1, DKK-1, OPG, RANKL, TRAP-5b, BAP and OPN were determined by an ELISA assay, while CEA, CA 15-3, TPA, CA 27.29, CYFRA 21-1, ICTP, PICP, PINP, PIIINP, PTHrP, IGF1, CT, OC, TNFαand IL-6 were assayed by radiometric methods. The clinicopathological characteristics and serum markers were compared among the subgroups identified either on the basis of the scan score (S1, S2, and S3) or of the disease outcome (A1-recession or stability, A2-deterioration). **Results**: S1 score was observed in 81 (52.94%) patients, S2 in 49 (32.03%) patients and S3 in the remaining 23 (15.03%) patients. A1 subgroup included 107 (69.93%) patients and 46 (30.07%) patients belonged to subgroup A2. IL-6, bcl-2 p53, MMP2 and OPG/RANKL ratio efficiently reflected the extent and severity of the initial skeletal involvement. TNFa, IL-6, p53, MMP2, and TRAP had the higher predictive strength being significantly lower in all measurements in patients with subsequent disease remission or stabilization. **Conclusion**: The proinflammatory cytokines IL-6 and TNFa help to predict more accurately BC+BM subgroups' clinical behavior.

P.B2.02.20

Gene expression in correlation to the cancer/testis antigen histone H2A family member B (H2AFB)

*L. I. Ohm, **M. S. Staege**;*
1Department of Surgical and Conservative Paediatrics and Adolescent Medicine, Halle, Germany.

The cancer/testis antigen H2AFB is a non-canonical histone variant of H2A. In our previous studies we observed high expression of H2AFB in Hodgkin lymphoma (HL) cells with very high expression in the chemotherapy resistant cell lines L-1236 and L-428. For further analysis of H2AFB we cloned H2AFB from Hodgkin lymphoma cells into a GFP fusion-protein expression vector. H2AFB-GFP showed an in-homogeneous enrichment in the nucleus of transfected cells. During mitosis H2AFB-GFP appeared to be partly associated with chromatin. H2AFB is known to be associated with transcriptional active chromatin. Therefore, we performed DNA microarray analysis of transgenic cells and identified H2AFB-regulated genes. Microarray data suggest that H2AFB over-expression induces an anti-apoptotic gene expression profile characterized by regulation of genes like programmed cell death protein 4 (PDCD4), BCL2 interacting protein 3 (BNIP3), Bcl-2-associated transcription factor 1 (BCLAF1; all down-regulated by H2AFB-GFP), and protein phosphatase 1 regulatory subunit 13 like (PPP1R13L; up-regulated by H2AFB-GFP). However, analyses of chemotherapy sensitivity showed no difference between H2AFB-transgenic cells in comparison to control cells. Similarly, transgenic expression of H2AFB-GFP had no impact on cell cycle progression. Interestingly, the expression of human endogenous retroviruses (HERV) families K and F is positively correlated with the expression of H2AFB in HL cells. Reactivation of HERV-like sequences in Hodgkin lymphoma has been described before. It seems likely that the high expression of the cancer/testis antigen H2AFB in HL cells and reactivation of endogenous retrovirus-like sequences are induced by a common mechanism leading to relaxation of gene regulation.

P.B2.02.21

The bridge between tumor-associated glycans and the anti-tumor immune response

***A. Zaal**, E. R. Li, J. Lübbers, Y. van Kooyk, S. J. van Vliet;*
VUmc university medical center, Amsterdam, Netherlands.

Many colorectal tumors display alterations in biosynthetic pathways of glycosylation, resulting in increased expression of specific tumor-associated glycan structures. Expression of these altered glycan structures is associated with metastasis and poor prognosis. Antigen presenting cells can recognize tumor-associated glycan structures, including the truncated O-glycan Tn antigen, via specific glycan receptors. Tn-antigen-mediated activation of the MGL receptor on dendritic cells, for example, induced regulatory T cells via the enhanced secretion of IL-10. Although these findings indicate that the expression of tumor-associated glycans modulates the anti-tumor immune response, the impact of these glycans on dendritic cells is still not fully understood. To investigate the impact of tumor-associated glycans on antigen presenting cells, RNA sequencing analysis was performed on human monocyte-derived dendritic cells stimulated with various tumor-associated glycans, including MGL ligands. GO term enrichment analysis of the differently expressed genes upon MGL ligation revealed an enrichment for genes involved in T cell differentiation. In addition, MGL ligation decreased gene expression of enzymes involved in glycolysis.
Since decreased glycolytic activity corresponds with a more regulatory function of antigen presenting cells, these data imply that MGL ligation reduces the anti-tumor immune response. Currently, we are further investigating the effect of MGL activation on metabolic changes in dendritic cells and subsequent T cell activation. Overall, our findings highlight the impact of tumor-associated glycans on anti-tumor immune responses and will increase our understanding of immune regulation by tumor-associated glycans.

P.B2.03 Environmental regulation anti-tumor responses - Part 3

P.B2.03.01

HHLA2 (B7H7) is highly expressed in human hepatocellular carcinoma cells and is associated with better patient survival

P. P. Boor[1], *K. Sideras*[1], *K. Biermann*[1], *J. Verheij*[2], *B. Takkenberg*[3], *S. Mancham*[1], *G. Zhou*[1], *Q. Pan*[1], *K. Tran*[1], *U. Beuers*[3], *T. M. van Gulik*[2], *J. N. IJzermans*[1], *M. J. Bruno*[1], *X. Zang*[4], *D. Sprengers*[1], *J. Kwekkeboom*[1];
[1]*ErasmusMC, Rotterdam, Netherlands,* [2]*University of Amsterdam, Amsterdam, Netherlands,* [3]*Tytgat Institute for Liver and Intestinal Research, Amsterdam, Netherlands,* [4]*Albert Einstein College of Medicine, New York, United States.*

Introduction: HHLA2 is a member of the B7-family and is thought to function predominantly as a T-cell co-inhibitory molecule. We assessed the expression of HHLA2 in hepatocellular carcinoma (HCC) and determined its relation to patient survival. Method: Tissue-microarrays with HCC tumors and tumor-free liver (TFL) tissues were immunohistochemically stained with an antibody against HHLA2 (clone: 566.1) and scored as negative, weak, intermediate, or strong expression. FACS-analysis of single cells isolated from freshly isolated tumors was used to further characterize HHLA2 expression. Results: In 27.8% of patients HHLA2-expression was absent on tumor cells, while 15.5% had weak expression, 37.1% had intermediate expression, and 19.6% had strong expression on tumor cells (n=194). Absent or weak tumor expression of HHLA2 was associated with poorer HCC-specific patient survival compared with intermediate or strong HHLA2 expression (average 72 versus 95 months; p=0.002). HHLA2 expression was predictive of HCC-specific survival independent of baseline clinicopathologic characteristics, like liver cirrhosis, alfa-feto protein serum level, tumor size, and number of lesions (HR 0.43; P=0.004). There was no association between HHLA2 expression in TFL tissues and patient survival. FACS analysis showed that HHLA2 was also expressed on $CD19^-$ $BDCA1^+$ myeloid dendritic cells and $CD14^+$ cells in tumors. Conclusion: Tumor cell expression of HHLA2 was observed in most HCC patients and is associated with better HCC-specific survival. HHLA2 expression in tumors may be induced in response to immunologic pressure, which may explain the positive association with prolonged survival.

P.B2.03.02

***CD5* and *CD6* expression levels as prognostic biomarkers for early-stage non-small cell lung cancer**

S. Casadó-Llombart[1], *F. Aranda*[1], *A. Moreno Manuel*[2], *S. Calabuig Fariñas*[2,3], *A. Herreros Pomares*[2], *S. Gallach-Garcia*[2], *I. Simoes*[1], *E. Carreras*[1], *M. Consuegra-Fernández*[1], *A. Blasco*[4], *A. Cunquero Tomas*[4], *M. Martorell*[3,5], *E. Jantus-Lewintre*[2,6], *C. Camps Herrero*[2,4,7], *F. Lozano*[1,8,9], *R. Sirera*[6];
[1]*Immunoreceptors of the Innate and Adaptative System, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBPAS), Barcelona, Spain,* [2]*Laboratorio de Oncología Molecular, Fundación para la Investigación, Hospital General Universitario de Valencia-CIBERONC, Valencia, Spain,* [3]*Departamento de Patología, Universitat de València, Valencia, Spain,* [4]*Servicio de Oncología Médica, Hospital General Universitario de Valencia-CIBERONC, Valencia, Spain,* [5]*Servicio de Anatomía Patológica, Hospital General Universitario de Valencia, Valencia, Spain,* [6]*Departament de Biotecnologia, Universitat Politécnica de València, Valencia, Spain,* [7]*Departament de Medicina, Universitat de València, Valencia, Spain,* [8]*Servei d'Immunologia, Centre de Diagnòstic Biomèdic, Hospital Clínic de Barcelona, Barcelona, Barcelona, Spain,* [9]*Departament de Biomedicina, Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain.*

Introduction: The study of the immune surveillance in the tumour microenvironment is leading to the development of new biomarkers and therapies. This research focuses on analysing *CD5* and *CD6* expression, two lymphocyte surface markers involved in TCR tuning, as potential prognostic biomarkers in resectable stages of Non-Small Cell Lung Cancer (NSCLC).
Materials and Methods:*CD5* and *CD6* gene expression were analysed by RTqPCR in 201 paired fresh frozen tumour and normal tissue samples of resected NSCLC. The Cancer Genome Atlas (TCGA) database was used to obtain an independent validation patient cohort. Prognostic value was assessed by Cox regression and Kaplan-Meier curves (log rank-test), considering significant $p < 0.05$.
Results: Local cohort consisted mainly of men, current or former smokers, with good performance status (PS=0). Patients with higher *CD5* expression had significantly increased overall survival (OS, 53.3 vs NR months, p = 0.011). Multivariate analysis allowed establishment of *CD5* expression as an independent prognostic biomarker for OS in early stages of NSCLC [HR=0.539; 95% CI, 0.329-0.883; p=0.014]. Further survival analysis of 97 patients from TCGA database, containing gene expression data for normal and tumoral tissue samples, confirmed high expression levels for both *CD5* and *CD6* as of prognostic value for relapse-free survival (34.98 vs 75.57 months, p=0.033; 25.31 vs 75.57 months, p=0.020, respectively) and OS (40.49 vs 77.97 months, p=0.038; 39.02 vs 77.97 months, p=0.034, respectively).
Conclusions: The present data support *CD5* expression level as a novel independent prognostic marker in resectable NSCLC.

P.B2.03.03

Identifying a novel role for fractalkine in T cell accumulation in the visceral adipose tissue of obesity-associated cancer patients.

M. J. Conroy[1], *A. Melo Rodriguez*[1], *S. Maher*[1], *S. L. Doyle*[2], *E. Foley*[1], *N. Ravi*[3], *J. V. Reynolds*[3], *A. Long*[1], *J. Lysaght*[1];
[1]*Trinity Translational Medicine Institute, Trinity College Dublin, Dublin, Ireland,* [2]*Dublin Institute of Technology, Dublin, Ireland,* [3]*St. James's Hospital, Dublin, Ireland.*

The global health burden of obesity continues to rise, resulting in increased incidence of associated morbidities. We have previously reported the importance of T cells in obesity-associated inflammation and demonstrated their active migration to the visceral adipose tissue (VAT) of patients with the obesity-associated malignancy, oesophageal adenocarcinoma (OAC). Furthermore, we have reported that chemokine receptor antagonism can significantly reduce such T cell migration to the VAT and may have therapeutic potential to ameliorate pathological inflammation in obesity and obesity-associated cancer. Here, we show that the inflammatory chemokine fractalkine (CX3CL1) is enriched in the VAT of OAC patients. Furthermore, our *ex vivo* demonstration of fractalkine-driven migration of OAC-derived T cells, suggests that this chemokine plays a role in T cell recruitment to VAT in OAC. Interestingly, surface expression of the fractalkine receptor CX3CR1 by high expressing circulating $CD8^+$ T cells is endocytosed but not degraded upon encountering fractalkine. We also show that such fractalkine-mediated endocytosis of CX3CR1 is accompanied by enhanced surface expression of ICAM-1 and L-Selectin on peripheral blood-derived $CD8^+$ T cells. Interestingly, our analyses identified these molecules in their soluble form among the most prevalent soluble adhesion molecules in the VAT of OAC patients suggesting that an abundance of fractalkine in VAT serves in T cell adhesion as well as T cell recruitment to this tissue in OAC patients. For the first time, these findings identify fractalkine as a potential therapeutic target to release inflammatory and cytotoxic T cells from the VAT and attenuate obesity-associated inflammation in OAC.

P.B2.03.04

Complete tumor desialylation drives tumor growth through hampered $CD8^+$ T cell cytotoxicity

L. A. M. Cornelissen, *A. Blanas, J. C. Van der Horst, L. Kruijssen, A. Zaal, T. O'Toole, Y. Van Kooyk, S. J. Van Vliet;*
VU University Medical Center, Amsterdam, Netherlands.

The tumor microenvironment is immunosuppressive, allowing tumor cells to escape from immune attack. Tumor cells generally display an aberrant glycosylation profile, amongst others characterized by an overexpression of sialylated structures. Sialic acids (Sias) are recognized by Siglec receptors, most of which are immune inhibitory receptors. Therefore, we hypothesized that Sias play a crucial role in suppressing anti-tumor immunity. Indeed, we have previously shown that Sia^{low} melanoma tumor cells, expressing reduced level of Sias on the cell surface, exhibited delayed *in vivo* growth due to an augmented effector T cell response. However, the role of Sia on other tumor types than melanoma and the effect of complete cancer cell desialylation on the anti-tumor immune response has never been studied. To generate Sia^{neg} glycovariants, we selected the mouse colorectal cancer cell line MC38 and desialylated the cells with the use of CRISPR/Cas9. Interestingly, the MC38-Sia^{neg} tumor cells displayed enhanced growth *in vivo* compared to their mock-transfected counterparts. Strikingly, MC38-Sia^{neg} tumors contained less $CD8^+$ T cells and these $CD8^+$ T cells had a reduced activation state. In an *in vitro* tumor killing assay MC38-Sia^{neg} tumor cells were less efficiently killed by activated cytotoxic T cells. Together our results indicate that a loss of Sias in colorectal cancer hampers $CD8^+$ T cell cytotoxicity. In conclusion, we show for the first time that complete desialylation drives tumor growth, which greatly impacts the design of novel cancer therapeutics aimed at targeting the tumor glycosylation profile.

P.B2.03.05

DNGR-1 as a Dendritic Cell-Specific Checkpoint in Antitumor Immunity

F. J. Cueto[1], *C. del Fresno*[1], *P. Brandi*[1], *A. Combes*[2], *A. R. Sánchez-Paulete*[3], *M. Enamorado*[1], *R. Conde-Garrosa*[1], *I. Melero*[4], *M. F. Krummel*[2], *D. Sancho*[1];
[1]*Spanish National Center for Cardiovascular Research, Madrid, Spain,* [2]*University of California San Francisco, San Francisco, United States,* [3]*Center for Applied Medical Research (CIMA), Pamplona, Spain,* [4]*IdISNA, CIMA and University Clinic, University of Navarra, Pamplona, Spain.*

Classical type 1 dendritic cells (cDC1s) are pivotal to antitumor immunity and their infiltration in tumors associates with better prognosis. DNGR-1 is a dead cell-sensing receptor highly restricted to cDC1s, but its role in antitumor immunity has not been clarified yet. Here, we found that DNGR-1 absence did not affect cross-presentation of tumor-associated antigen, tumor growth or responsiveness to anti-PD-1 treatment. However, Flt3L-expressing B16 melanoma showed delayed tumor growth in DNGR-1-deficient mice. Indeed, treatment of mice with systemic Flt3L in DNGR-1-deficient mice led to improved antitumor immunity. Enhanced antitumor immunity in the absence of DNGR-1 was T cell-dependent and correlated with increased infiltration of cDC1s within B16F10 tumors. Absence or blockade of DNGR-1 in the presence of Flt3L resulted in increased expression of CCR7 on cDC1s but not on cDC2s. These results correlated with the analysis of TCGA data from patients with different cancers, which indicates a strong association between the expression of CCR7 ligands CCL19 and CCL21 with cDC1 infiltration. Our data show that blockade of DNGR-1 signaling promotes cDC1 infiltration within tumors in the presence of Flt3L, suggesting CCR7 upregulation as a potential mechanism.

P.B2.03.06

Exploring the effectsof systemic and local immunity on tumour regression grade in oesophagealadenocarcinoma

***M. R. Dunne**, E. K. Foley, J. V. Reynolds, J. O'Sullivan;*
Trinity Translational Medicine Institute, Dublin, Ireland.

Oesophageal adenocarcinoma (OAC) is an aggressive malignancy, with a 5 year survival of <15%, and incidence is rapidly rising in the Western world. Although multi-modal neoadjuvant chemotherapy and chemoradiotherapy (neo-CT) approaches have significantly improved survival outcome, this only occurs for a minority of patients, and it is currently not possible to predict which patients will respond to treatment.
This study explored the association between 54 circulating immune, vascular and angiogenic markers, and patient response to neo-CT treatment. Serum was taken from n=80 treatment-naive OAC patients levels of circulating markers were quantified using multiplex ELISA technology. Markers assessed encompassed key inflammatory cytokines, chemokines, angiogenic factors and markers of vascular injury. The concentration of circulating markers was compared with tumour regression score (Mandard grade 1-5), collapsed into a 3 tier system.
Levels of CCL4, a chemoattractant for natural killer cells and monocytes, were significantly lower in patients with a poor response to treatment ($p<0.001$), while levels of angiogenic factor Tie-2 were significantly higher in poor responders ($p<0.05$), when compared to patients who experienced complete or partial responses. Future work will compare these circulating marker profiles with other clinical features, e.g. survival time, tumour stage, nodal status and level of immune cell infiltration into tumour biopsies.

P.B2.03.07

Mechanism of the effects of cancer associated fibroblasts on the functional changes observed in T cells

***G. Gunaydin**, D. Guc;*
Hacettepe University Cancer Institute, Ankara, Turkey.

Fibroblasts, turn into cancer associated fibroblasts (CAFs) and myofibroblasts in the tumor microenvironment. Tissue fibroblasts have previously been shown to affect T lymphocyte functions. CAFs were reported to show immunosuppressive effects similar to fibrocytes. They also display similar phenotypic and functional characteristics to the circulating fibrocytes, which were reported to represent a unique MDSC subset. However, studies investigating the underlying mechanisms responsible for such effects of CAFs on T cells are limited in the literature. The scope of this study is to determine the role of tumor stromal fibroblasts on the alterations in T cell effector functions. For this reason, CAFs were isolated from tumors generated by a rat chemical breast carcinoma model.
PBMCs were cocultured with either normal-fibroblasts or CAFs and proliferations of PBMCs were assessed by CFSE assays. Surface and intracellular expressions of immune activation markers of T lymphocytes cocultured with CAFs were analyzed with flow cytometry. Breast cancer associated CAFs were shown to decrease proliferations of splenocytes in cocultures. CAFs also decreased the protein expressions of activation markers of T cells. In addition, splenocytes cocultured with CAFs were found to have decreased expression levels of genes associated with cellular activation and effector functions. The alterations in T cells in terms of gene and protein expression levels have been shown via phenotypical and functional analyses. This study helps elute the mechanisms of the effects of cancer associated fibroblasts on the functional changes observed in T cells in the tumor microenvironment.

P.B2.03.08

V-set and immunoglobulin domain-containing 4 (VSIG4) expressed on macrophages suppressed anti-tumor immune responses in myelodysplastic syndromes

***M. Ishibashi**[1], H. Tamura[2], I. Choi[3], H. Takahashi[1];*
[1]Department of Microbiology and Immunology, Nippon Medical School, Tokyo, Japan, [2]Department of Hematology and, Nippon Medical School, Tokyo, Japan, [3]Department of Microbiology and Immunology, Inje University College of Medicine, Busan, Korea, Republic of.

Introduction: Myelodysplastic syndromes (MDS) are a group of hematologic malignancies, and MDS blast cells increase clonal proliferation during the disease-progression process, while immune cells in the bone marrow microenvironment become less efficient. V-set and immunoglobulin domain-containing 4 (VSIG4) molecule is a new B7 family related protein and a strong negative regulator of T-cell proliferation. However, the role of VSIG4 in tumors remains unknown. Thus we investigated the expression and functions of VSIG4 in MDS. **Methods:** The expression of VSIG4 was analyzed using flow cytometry (FCM) in MDS cell lines and bone marrow samples from patients. The proliferative potential was examined by BrdU incorporation using FCM. The antibody-dependent cellular cytotoxicity (ADCC) of a natural killer (NK) cell line was determined by measuring LDH activity. The production of IFN-γ from donor T cells was measured in cell culture supernatants using ELISA. **Results:** Cell-surface VSIG4 expression was highly expressed on macrophages, especially tumor-associated macrophages, but not on $CD34^+$ blasts (tumor cells) in patients with MDS and AML transformed from MDS (AL-MDS). Furthermore, high expression of VSIG4 was detected on monoblasts (tumor cells) from patients with chronic myelomonocytic leukemia. $VSIG4^+$ MDS cells had higher proliferative potential than $VSIG4^-$ cells. CD45-mediated ADCC activity of NK cells was inhibited in VISG4-expressing MDS cells in comparison with controls. Moreover, the production of IFN-γ from donor T cells co-cultivated with $VISG4^+$ MDS cells was decreased compared with $VISG4^-$ cells. **Conclusions:** Our results revealed that VSIG4 expressed on macrophages and monoblasts suppresses antitumor immune responses.

P.B2.03.09

Phenotypic and functional analysis of peripheral blood, healthy mucous and tumour lymphoid populations of patients with gastric adenocarcinoma

***I. Juarez**[1], A. Gutierrez-Calvo[2], A. Blazquez[2], E. Ovejero[2], I. Laso[2], A. Lopez[2], R. Gomez[2], J. M. Martin-Villa[1];*
[1]Universidad Complutense de Madrid, Dpt. of Immunology, Madrid, Spain, [2]Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Madrid, Spain.

The presence of infiltrating lymphocytes in the tissue is a good prognostic factor in gastric cancer, although there are few functional data.
We studied PBL, as well as healthy and tumoral mucose lymphocytes (HML/TML) of 6 patients with gastric adenocarcinoma. Tissue explants were grown for 20 days in cDMEM with 1000 U/ml IL-2, and HML and TML were thus obtained.
PBL showed a significant increase in PD-1+ cells in gastric cancer patients compared to controls (27.5% and 11.6%, respectively, $p<0.0001$). No differences were found in the number of CD8+ cells, although CD8 expression was significantly lower in gastric cancer patients' PBL (392.8) compared to controls (625.1; $p=0,0169$). A higher frequency of PD1+ cells were found at a higher frequency in TMLs and HMLs than PBL (52.9% $p<0.01$).
In patients, a large dispersion in TML, but not HML, CD8+ cells were found (27.7%±11.8; 41.8%±3.3). FCM data were confirmed by IHC CD8 staining. These differences may be due to a defect in the IFN-γ mediated CD8 cell recruitment and activation within the tumor environment.
Lymphocytes were stimulated with PMA-ION and the production of IFN-γ was quantified by FCM. In two of the three patients analyzed, detectable amounts of IFN-γ were found, either in PBL-CD8+, HML-CD8+ or HML-CD8+. In the patient in whom no TML-CD8+ were detected, the number of CD8+ cells producing IFN-γ in PBL and LMS is very low (18.1% and 1.9% respectively). These data suggest that intrinsic defects in IFN-mediated activation-γ may limit CD8 cell response in gastric cancer.

P.B2.03.10

Increased *Δ133TP53β* in glioblastoma: an alternative to p53 mutation in promoting brain tumor progression

***M. Kazantseva**[1,2], R. A. Eiholzer[1], S. Mehta[1,2], A. Taha[3], S. Bowie[1], I. Roth[1], J. Zhou[1,3], S. M. Joruiz[4], M. A. Baird[1], N. A. Hung[1], T. L. Slatter[1,2], A. W. Braithwaite[1,2];*
[1]University of Otago, Dunedin, New Zealand, [2]Maurice Wilkins Centre for Molecular Biodiscovery, Auckland, New Zealand, [3]Southern District Health Board, Dunedin, New Zealand, [4]University of Dundee, Dundee, United Kingdom.

The p53 (*TP53* gene) is a powerful suppressor of cancer. In contrast, the p53 isoform, Δ133p53 is increased in cancers that are more aggressive and those with a poorer response to treatment. As Δ133p53 isoforms have tumor promoting migration and inflammatory properties this study investigated if Δ133p53 contributed to brain tumor (glioblastoma) progression.
The expression of *full-length TP53* and six *TP53* mRNA variants were quantitated by real time-quantitative PCR in 89 glioblastomas and correlated with tumor associated macrophage content, various other immune cell markers and whether the tumors had wild-type or mutant p53. Hypoxic areas in glioblastoma tissue were detected with carbonic anhydrase nine staining and *Δ133TP53β* expression using RNAscope. To determine if Δ133p53β could contribute to the temozolomide resistance and/or promote cell survival in response to oxidative stress, 10.1 cells expressing a murine 'mimic' of Δ133p53 (Δ122p53) were treated with temozolomide or *tert*-butyl hydroperoxide, respectively.
Elevated levels of *Δ133TP53β* mRNA characterized glioblastomas with increased CD163 positive macrophages and wild-type p53. We found *Δ133TP53β* expression was localized to cancer cells in areas with increased hypoxia, and in stromal cells with *CCL2* expression. Tumors with increased *Δ133TP53β* had increased numbers CSF1R and PDL1 positive cells. In addition, cells expressing Δ122p53 were resistant to temozolomide treatment and oxidative stress; suggesting Δ133p53β could reduce the sensitivity to temozolomide and promote cell survival under oxidative stress.
We show elevated *Δ133TP53β* aids tumor progression by promoting an immunosuppressive and chemoresistant environment. This study suggests a role for hypoxia signalling in the regulation of *Δ133TP53β* expression.

P.B2.03.11

In vivo suppression of murine tumour growth through CD8+CTL via activated DCs by sequential administration of alpha-galactosylceramide

H. Kogo[1,2,3]*, M. Shimizu*[1]*, Y. Negishi*[1]*, H. Takahashi*[1]*;*
[1]*Department of Microbiology and Immunology, Nippon Medical School, Tokyo, Japan,* [2]*Department of Gastrointestinal and Hepato-Biliary-Pancreatic Surgery, Nippon Medical School, Tokyo, Japan,* [3]*Department of Surgery, Kitamurayama Hospital, Yamagata, Japan.*

Tumour immunity is largely attributed the effective priming and activation of tumour-specific class I MHC molecule-restricted CD8+ CTLs. CD205+ DCs can cross-present the epitopes of captured tumour antigens associated with class I MHC molecules alongside co-stimulatory molecules to prime and activate tumour-specific CD8+ CTLs. Immunosuppressive tolerogenic DCs with reduced co-stimulatory molecules may be a cause of impaired CTL induction. Hepa1-6-1 cells were established from the murine hepatoma cell line Hepa1-6; these cells grow continuously after subcutaneous implantationinto syngeneic B6 mice and do not prime CD8+ CTLs. In thisresearch, we show that the growth of ongoing tumours was suppressed by activated CD8+ CTLs with tumour-specific cytotoxicity through the administration of alpha-GalCer, which is a glycolipid known to stimulate iNKT cells and selectively activate CD205+ DCs. Moreover, we demonstrated thatsequential repetitive intraperitoneal inoculation with alpha-GalCer every 48 hours appeared to convert tolerogenic CD205+ DCs into immunogenic DCs with a higher expression of co-stimulatory molecules and a stronger cross-presentation capacity, which primed CTL precursors and induced tumour-specific CD8+ CTLs within the tumour environment without activating iNKT cells. These findings provide a new method for cancer immunotherapy to convert tolerogenic CD205+ DCs within tumours into immunogenic DCs through the sequential administration of an immuno-potent lipid/glycolipid, and then activated immunogenic DCswith sufficient expression of co-stimulatory molecules prime and activate tumour-specific CD8+ CTLs within the tumour to suppress tumour growing.

P.B2.03.12

Abrogation of the immunosuppressive tumor microenvironment in cholangiocarcinoma by targeting PD-1 or GITR

G. Zhou, D. Sprengers, R. Erkens, S. Mancham, M. Doukas, L. Noordam, P. P. Boor, R. W. van Leeuwen, B. Groot Koerkamp, W. G. Polak, J. de Jonge, J. N. IJzermans, M. J. Bruno, ***J. Kwekkeboom****;*
Erasmus MC - University Medical Centre, Rotterdam, Netherlands.

Cholangiocarcinoma (CCA) is an aggressive malignancy of the biliary tract. CCA-patients generally present with advanced disease for which no curative treatment is available. Whether CCA is responsive to immune checkpoint antibody therapy is unknown, and little is known about the tumor immune microenvironment of CCA. We characterized tumor-infiltrating lymphocytes (TIL) isolated from freshly resected CCA tumors, determined their expression of co-signaling molecules, and assessed the effects of targeting these molecules on TIL functions in *ex vivo* assays. TIL contained lower proportions of CD8+ T cells, NKT cells and NK cells and higher proportions of CD4+Foxp3+ regulatory T cells (Treg) than lymphocytes isolated from tumor-free liver tissues (TFL) of the same patients. Immunohistochemistry showed that the majority of CD8+ and CD4+ T cells were sequestered at the tumor margin, while Treg accumulated in the tumors. Tumor-infiltrating CD8+ T cells showed reduced expression of the cytotoxic molecules perforin and granzyme compared to those in TFL and blood.
Co-stimulatory receptor GITR as well as co-inhibitory receptors PD-1 and CTLA4 were over-expressed on tumor-infiltrating T cells compared with T cells in TFL and blood. PD-L1, CD86 and CD80 were expressed on antigen-presenting cells in tumors, but GITR ligand not. Antagonistic targeting of PD-1 with nivolumab or agonistic targeting of GITR with GITR-ligand enhanced granzyme B and effector cytokine production and/or T cell proliferation in *ex vivo* stimulations of TIL with CD3 and CD28 antibodies. **Conclusions:** The tumor microenvironment in CCA is immunosuppressive. PD-1 and GITR are potentially promising targets for immunotherapy of CCA patients.

P.B2.03.13

Bone marrow endothelial cells sustain a tumor-specific CD8$^+$T cell subset with suppressive function in myeloma patients

P. Leone*, G. Di Lernia, D. Giannico, A. G. Solimando, A. Vacca, V. Racanelli;*
University of Bari Medical School, Bari, Italy.

Endothelial cells (EC) line the bone marrow microvasculature and are in close contact with CD8$^+$ T cells that come and go across the permeable capillaries. Because of these intimate interactions, we investigated the capacity of EC to act as antigen-presenting cells (APC) and modulate CD8$^+$ T cell activation and proliferation in bone marrow of patients with multiple myeloma (MM) and monoclonal gammopathy of undetermined significance. We found that EC from MM patients show a phenotype of semi-professional APC given that they express low levels of the co-stimulatory molecules CD40, CD80 and CD86, and of the inducible co-stimulator ligand (ICOSL). In addition, they do not undergo the strong switch from immunoproteasome to standard proteasome subunit expression which is typical of mature professional APC such as dendritic cells. EC can trap and present antigen to CD8$^+$ T cells, stimulating a central memory CD8$^+$ T cell population that expresses Foxp3 and produces high amounts of IL-10 and TGF-β. Another CD8$^+$ T cell population is stimulated by professional APC, produces IFN-γ, and exerts antitumor activity. Thus, two distinct CD8$^+$ T cell populations coexist in the bone marrow of MM patients: the first population is sustained by EC, expresses Foxp3, produces IL-10 and TGF-β, and exerts pro-tumor activity by negatively regulating the second population. This study adds new insight into the role that EC play in MM biology and describes an additional immune regulatory mechanism that inhibits the development of antitumor immunity and may impair the success of cancer immunotherapy.

P.B2.03.14

MESENCHYMAL STROMAL CELL SIALYATION ENHANCES T LYMPHOCYTE IMMUNE SUPPRESSION

K. Lynch[1]*, M. O'Dwyer*[1]*, A. Ryan*[1,2]*, T. Ritter*[1]*;*
[1]*Regenerative Medicine Institute (REMEDI), College of Medicine, Nursing & Health Sciences, National University of Ireland, Galway, Galway, Ireland,* [2]*Discipline of Pharmacology, College of Medicine, Nursing & Health Sciences, National University of Ireland, Galway, Galway, Ireland.*

Introduction: Little is known about the mechanisms of immune modulation mediated by mesenchymal stromal cells (MSC) in the tumour micro-environment (TME). Aberrant glycosylation is a hallmark of cancer cells, playing an important role in tumour progression. Here we sought to investigate if regulation of MSC sialylation alters their ability to inhibit T-cell function in an inflammatory micro-environment, characteristic of the TME.**Methods:**MSC were treated with both tumour necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) (i-MSC) for 72 hours and the sialic acid levels were analysed by flow cytometry. MSC and i-MSC were co-cultured in MSC-lymphocyte co-cultures for 96 hours. T-cell proliferation, activation, death and differentiation were determined by flow cytometry. Both MSC and i-MSC were pre-treated with a sialyltransferase inhibitor (3FaxNeu5Ac) for 72 hours prior to TNF-α and IL-1β stimulation and co-cultured with lymphocytes. **Results:**i-MSC displayed phenotypical changes. i-MSC displayed increased levels of both α2-3/α2-6 linked sialic acid when compared to MSC. i-MSC displayed an enhanced ability to inhibit the proliferation of lymphocytes when compared to MSC alone. i-MSC inhibited the proliferation of stimulated CD4+/CD8+ lymphocytes. Following sialyltransferase inhibition, i-MSC lost the ability to suppress both CD4+/CD8+ lymphocytes resulting in significant restoration of lymphocyte proliferation. Sialic acid expression on the cell surface correlated with both CD4+/CD8+ lymphocyte suppression.**Conclusions:** Our findings confirm that inflammation induces MSC sialylation and enhances their ability to suppress activated lymphocytes. We suggest that understanding the importance of MSC sialylation is likely to lead to the identification of a new molecular target.

P.B2.03.15

Clinical relevance of intratumoral dendritic cells in neuroblastoma

O. Melaiu[1,2]*, M. Chierici*[3]*, V. Lucarini*[1]*, M. Compagnone*[1]*, G. Zicchedda*[1]*, G. Jurman*[3]*, R. Boldrini*[1]*, C. Furlanello*[3]*, F. Locatelli*[1]*, D. Fruci*[1]*;*
[1]*Ospedale Pediatrico Bambino Gesù, Rome, Italy,* [2]*University of Pisa, Pisa, Italy,* [3]*Fondazione Bruno Kessler, Trento, Italy.*

Introduction: The prognostic value of tumor-infiltrating T lymphocytes (TILs) has been demonstrated in several human cancers. Recently, we have shown that TILs have a prognostic value greater than, and independent of the criteria currently used to stage neuroblastoma. We defined an immunoscore based on the presence of different T-cell subsets that associates with favorable clinical outcome in MYCN-amplified tumors. We also demonstrated that the combined PD-L1 and HLA class I tumor cell density represents a novel prognostic biomarker for neuroblastoma patients. Here we sought to further dissect the neuroblastoma microenvironment, evaluating density of infiltrating dendritic cells (iDC), macrophages and NK cells. Moreover, we tested whether immune gene profiling of neuroblastoma could identify novel prognostic and therapeutic targets for these patients.
Methods: *In situ* immunohistochemical staining for CD141, CD163 and NKp46 was assessed in 104 neuroblastoma specimens and correlated with clinical outcome. Gene expression profiling using Nanostring nCounter Immune panel was also performed in 36 neuroblastoma samples. Publically available datasets were used to validate the results.
Results: High density of iDCs, macrophages and NK cells was correlated with the presence of TILs, tumor HLA class I expression and favorable clinical outcome, suggesting their potential role in controlling tumor development. These data were confirmed by gene expression profiling analyses. Clustering analysis revealed the existence of distinct expression profiles in high- and low-risk neuroblastomas.
Conclusions: These results may provide a rationale for improving risk stratification of patients and addressing towards a more targeted therapy.

P.B2.03.16

The balance between activated follicular helper T-cells and follicular regulatory T-cells infiltrating human breast cancer guides anti-tumor immune responses

G. Noel[1], *M. Langouo*[1], *S. Garaud*[1], *G. Van den Eynden*[2], *A. Boisson*[1], *H. Duvillier*[3], *D. Larsimont*[4], *K. Willard-Gallo*[1];
[1]*Molecular Immunology Unit-Institut Jules Bordet-ULB, Bruxelles, Belgium,* [2]*Anatomical Pathology Department-GZA Hospital Sint-Augustinus, Antwerp, Belgium,* [3]*Flow Cytometry Core Facility-Institut Jules Bordet-ULB, Bruxelles, Belgium,* [4]*Anatomical Pathology Department-Institut Jules Bordet-ULB, Bruxelles, Belgium.*

The recent success of immunotherapy highlights the importance of then immune response in cancer treatment. In human breast cancer (BC), tumor infiltrating lymphocytes (TIL) can organize in tertiary lymphoid structures (TLS) in the stroma. We have shown that CXCL13, a B-cell chemoattractant, is involved in TLS formation and associated with positive clinical outcomes. The present study investigated how TLS functionally contributes to immune responses in human BC.
We used fresh primary breast tissues to prepare primary tumor supernatants for immunoglobulin analysis and TIL for flow cytometric analysis and sorting. Matched formalin-fixed paraffin-embedded tissues were used for TIL and TLS scoring and organization.
CXCR5, the CXCL13 receptor, is expressed on infiltrating B-cells, $CD4^+$ T-cells [follicular helper T (Tfh) cells] and interestingly a $CD8^+$ T-cell subpopulation. All of these $CXCR5^+$ TIL co-localize in TLS, but only $ICOS^+PD\text{-}1^+$ Tfh TIL express high levels of *BCL6*, *IL21*, *CXCL13* and *IFNG* mRNA and functionally help B-cell differentiation *in vitro*. $ICOS^+PD\text{-}1^+$ Tfh TIL are also correlated with activated $CD8^+$ TIL in tumor tissues and IgG in tumor supernatants. Follicular regulatory T-cells (Tfr), express GARP, a sign of active TGFβ, within TLS. The Tfh/Tfr ratio is significantly correlated with IgG but also with IgA and IgM production suggesting that the balance between effector and regulatory Tfh TIL influence TLS activities.
Activated $ICOS^+PD\text{-}1^+$ Tfh and $GARP^+$ Tfr TIL are major players in TLS functionality, regulating anti-tumor immune responses and likely playing an important role in the response to immunotherapy.

P.B2.03.17

Stromal cell PD-L1 inhibits CD8 T cell anti-tumour immune response and promotes colon cancer

G. O'Malley[1], *O. Treacy*[1], *K. Lynch*[1], *S. Naicker*[1], *P. Lohan*[1], *P. Dunne*[2], *T. Ritter*[1], *L. J. Egan*[1], ***A. E. Ryan***[1];
[1]*National University of Ireland, Galway, Galway, Ireland,* [2]*Queens University Belfast, Belfast, Ireland.*

Stromal cells of mesenchymal origin reside below the epithelial compartment and provide structural support in the intestine. These intestinal stromal cells interact with both the epithelial cell compartments as well as infiltrating hematopoietic immune cells, however, little is known about their function and phenotype in the inflammatory tumour microenvironment. Using a syngeneic immunogenic colorectal cancer model, we show that TNF-α initiated inflammatory signalling in CT26 colorectal cancer cells selectively induces PD-L1 expression in stromal cells. Stromal cell PD-L1 potentiates enhanced immunosuppression, characterised by inhibition of $CD8^+$ granzyme B-secreting T cells and, consequently, enhanced tumour progression. To confirm a definitive role for stromal cell PD-L1 in the suppression of T cell proliferation and activation, we targeted the PD-1/PD-L1 signalling axis using a monoclonal blocking antibody to the PD-1 receptor. We observed high PD-1 expression on CD4+ and CD8+ T cells and treatment with anti-PD-1 antibody significantly reduced inflammatory tumour conditioned stromal cells ability to suppress the proliferation of CD8+ T cells. Additionally, PD-1 blockade was sufficient to restore lymphocyte activation and cytolytic potential, as measured by IFN-γ, TNF-α and granzyme B secretion. *In vivo*, treatment with anti PD-1 reversed stromal cell-mediated inhibition of CD8+ T cell cytolytic capacity and this was associated with increased tumour volume and invasive potential. We validated these findings in human stromal cells in the inflammatory tumour microenvironment. Collectively these results demonstrate the critical role of the PD-1/PD-L1 signalling axis in the ability of tumour-conditioned stromal cells to inhibit CD8+ T cell mediated anti-tumour immune effector functions

P.B2.03.18

The Model Regarding Participation of the Immune System in Metastatic Spread

D. Sepiashvili;
Oncology Dispensary LTD, Tbilisi, Georgia.

Cancer cells (CC) appearing in a macrophage, may not die because the process of apoptosis is disrupted in them. In the case of "incomplete phagocytosis", concealed in a macrophage CC can move towards the direction of lymphatic capillaries or directly to the blood vessels. The phagocyte with CC freely penetrates into the capillaries, reaches the lymph nodes. CC can multiply in the transport macrophage. After reaching a lymph node, the phagocyte, with incubated CC in it, is dying, insemination of CC occurs in the lymph nodes, and the new cycle is put into action.
The sizes of the microorganisms mainly vary within the range 1 - 4 μm. Most zooblasts have the diameter of 10-20 μm. and rarely are changed more than 2 times beyond this range. The sizes of macrophages equal 20-80 μm. Most researchers, considering the sizes of cells, indicate that the stem cells are much smaller in size, than more complete and highly proliferative cells. It turns out, that the sizes of the microbes and stem cells are of the same kind and comparable.
Macrophages are much more actively phagocytize microobjects with a size of 1-4 μm. Perhaps, with small-sized cancer stem cells, the special "tropism" of macrophages to the latter, is explained. Perhaps, this is the reason for the steady progression of tumor growth.
If this corresponds to reality then our ideas on the mechanisms of progression and dissemination of tumors must undergo serious revision. It is necessary to find the ways immunotherapy of "incomplete phagocytosis".

P.B2.03.19

The effect of radiofrequency ablation on the frequency of CD4+ T cells in patients with inoperable pancreatic cancer

N. Toria[1], *M. Mizandari*[2], *N. Kikodze*[3], *I. Pantsulaia*[3], *N. Janikashvili*[1], *T. Chikovani*[1];
[1]*Department of Immunology, Tbilisi State Medical University, Tbilisi, Georgia,* [2]*Department of Interventional Radiology, Tbilisi State Medical University, Tbilisi, Georgia,* [3]*Institute of Medical Biotechnology, Tbilisi State Medical University, Tbilisi, Georgia.*

Introduction: There is an increased interest in radiofrequency ablation (RFA) as the new type of local thermal ablative therapy for inoperable pancreatic cancer. In addition to cancer eradication, RFA favors tumor antigen release followed by the increase in specific anti-tumor immune response. However, this effect lasts for short time period. We suppose that it will be able to maintain anti-tumor immune response with repeatable RFA. The aim of the study was to explore the impact of repeated intraluminal RFA on the frequency of CD4+ T cells in patients with inoperable pancreatic cancer.
Methods: Patients with inoperable pancreatic cancer underwent three repetitive RFA procedures followed by self-expanding stent placement. Peripheral blood was obtained after one month of each procedure. Healthy age-matched volunteers were used as controls. The percentages of $CD39^+$ cells were separately quantified within $CD4^{low}$ and $CD4^{high}$ populations. Data were acquired on a FacsArray cytometer and analyzed with FlowJo® v7.5.6 software.
Results: Our results demonstrate that the frequency of total circulating $CD4^+$T lymphocytes was comparable between the patients and controls. After one month of RFA procedure, the frequencies of $CD4^{total}$ and $CD4^{+\,low}$ T cells were increased. The percentage of $CD39^+$ cells was decreased after repeated RFA procedure.
Conclusion: It is the first time to study the effect of repetitive ablation on adaptive immune response and may, therefore, uncover an important new target for therapeutic intervention as well as relevant treatment of this disease.
This research was funded by Shota Rustaveli National Science Foundation (Grant №: PhD_F_17_46).

P.B2.03.20

The downregulation of PDCD4 induced by progesterone is mediated by PI3K/ Akt/mTOR signaling pathway in human endometrial cancer cells

W. Zengtao[1], *X. Wang*[1,2];
[1]*Department of Gynecology and Obstetrics, Clinical Medical School, Jinan, China,* [2]*Department of Immunology, School of Basic Medical Sciences, Shandong University, Jinan, China.*

The endometrium is regulated by changing concentrations of ovarian hormones, such as estrogen and progesterone, and shows periodical changes. Apoptosis-related gene *programmed cell death 4* (*Pdcd4*) is identified as a tumor suppressor gene that inhibits neoplastic transformation, tumor progression, and translation. It has been reported that multiple factors participate in regulation of PDCD4 mRNA and protein. This study aims to explore the effect and mechanism of estrogen or progesterone on PDCD4 mRNA and protein expression in human endometrial cancer cells. We demonstrated that progesterone could effectively decrease the expression of PDCD4 protein and PI3K/AKT/mTOR pathway may be involved in the downregulation of PDCD4 protein. In conclusion, these results suggest that the downregulation of PDCD4 induced by progesterone could affect the therapeutic efficacy of progesterone in human endometrial cancer or endometriosis, which may have important implications for progesterone treatment in clinic.

P.B2.03.21

Targeting lysyl oxidase (LOX) favors T cell migration in the tumor stroma

A. NICOLAS-BOLUDA[1,2], *J. Vaquero*[3], *S. Barrin*[1], *C. Kantari-Mimoun*[1], *G. Renault*[1], *A. K. Silva*[2], *L. Fouassier*[3], *F. Gazeau*[2], *E. Donnadieu*[1];
[1]*Institut Cochin, Paris, France,* [2]*Laboratoire Matière et Systèmes Complexes, Paris, France,* [3]*Centre de Recherche Saint-Antoine, Paris, France.*

In the last decade, there has been an intense development of immunotherapeutic strategies boosting T cells with efficient anti-tumor activities. These include the use of monoclonal antibodies against the immunosuppressive surface molecules such as CTLA-4 and PD-1. However, complete and durable responses are only seen in a fraction of cancer patients. One of the determinants in the success of T cell-based immunotherapies lies on the ability of effector T cells to migrate within the tumor and access its specific antigens. Solid tumors are characterized by an aberrant organization of the extracellular matrix (ECM) in the form of highly reticulated and long linear collagen fibers, which has been shown to affect T cells penetration into tumor islets.

There are currently many strategies in development that target tumor ECM including the inhibition of lysyl oxidase, an extracellular copper-dependent enzyme upregulated in many tumors that catalyzes the cross-linking of collagen. Here, using a subcutaneous model of human biliary duct carcinoma (EGI-1) and dynamic imaging on fresh tissue slices, we investigated the consequences of LOX inhibition on the intratumoral migration of T lymphocytes. Our data indicates that LOX inhibition with beta-aminopropionitrile induced a significant decrease in tumor stiffness mapped using shear wave elastography (SWE) that correlates with the increase of the ability of T cell to migrate within the tumor. These experiments support the rationale of combining collagen-degrading strategies with approaches boosting T cells such as anti-PD-1 antibodies.

P.B2.03.22

Novel potential target genes attract the attention on T regulatory cells in malignant mesothelioma

S. Oliveto[1,2], *A. Miluzio*[1], *N. Manfrini*[1], *P. Gruarin*[1], *S. Curti*[1], *L. Mutti*[3], *M. R. Benvenuti*[4], *P. Novellis*[5], *G. Veronesi*[5], *M. Pagani*[1,2], *S. Biffo*[1,2];
[1]*INGM, MILANO, Italy,* [2]*University of Milan, Milan, Italy,* [3]*University of Salford, Manchester, United Kingdom,* [4]*Thoracic Surgery Unit, ASST Spedali Civili, Brescia, Italy,* [5]*Humanitas Clinical and Research Centre, Rozzano, Italy.*

Characterization of tumor infiltrating lymphocytes (TILs) is crucial for understanding the mechanisms of cancer progression and immunotherapy reaction. Blockade of immune checkpoints, such as inhibition of CTLA4 and PD-L1, is the master approach to enhance antitumor immunity. Unfortunately, durable responses fail in most patients, suggesting the persistence of immunosuppressive mechanisms. CD4+CD25+Foxp3+ tumor-infiltrating T regulatory cells (Tregs) are responsible for the suppression of effector T cells and, as a consequence, they favour the tumor in escaping the immune defence. Here we show that Mesothelioma tumors present lymphocyte infiltrates containing proliferating cells. Foxp3+ cells are specifically organized in the infiltrates' periphery andhave extremely high immunosuppressive capabilities. Thus, we isolated intratumoral CD4+ Tregs from surgical chemonaive MPM samples and performed RNA sequencing analysis in order to characterize their molecular signature. Tregs infiltrating MPM upregulated 1) several immune checkpoint genes, such as OX-40, TIGIT and TIM-3, as in other tumors and 2) a specific set of cytokines and citokine receptors. Surprisingly, they expressed also a set of genes never described before which underlines the uniqueness of the immunosuppression in MPM. In order to identify whether immunosuppression is driven by tumor-derived signals, we are now performing topological positioning analysis of Tregs in MPM tumors. Targeting specific Treg cells by manipulating tumor-derived signals may become a novel approach for MPM treatment.

P.B2.04 Environmental regulation anti-tumor responses - Part 4

P.B2.04.01

Hodgkin lymphoma secreted factors support differentiation of monocytes into macrophages with enhanced endocytic activity

A. Arlt[1], *F. von Bonin*[1], *T. Pukrop*[2], *J. Wilting*[3], *L. Trümper*[1], *D. Kube*[1];
[1]*Clinic for Hematology and Medical Oncology, Göttingen, Germany,* [2]*Clinic for Internal Medicine III, Regensburg, Germany,* [3]*Institute of Anatomy and Cell Biology, Göttingen, Germany.*

Hodgkin lymphoma (HL) is a hematological malignancy characterized by a rich and complex tumor microenvironment (TME). The malignant Hodgkin-Reed-Sternberg (HRS) cells account for less than 1% of the disease-related cells and are embedded in a reactive background of immune infiltrates. Hence, HRS cells highly depend on signaling cross-talk with non-transformed neighboring cells. Tumor-associated macrophages (TAMs) have been found to promote tumor development by promoting proliferation, angiogenesis and suppressing anti-tumor immune responses. In HL the number of TAMs was found to correlate with poor prognosis. Therefore, the aim of our study is the characterization of interactions between HRS cells and macrophages.
We found that *in vitro* HL cells secrete factors can attract and differentiate primary human monocytes into macrophages. Phenotypic characterization revealed that these macrophages resemble a M2 phenotype with a strong expression of CD206 and adhesion markers. Functional studies further showed that these features are accompanied with an increased endocytosis of CD206 specific targets. Additionally, we observed a high MMP-9 secretion and an altered tumor formation in an *in ovo* xenograft model suggesting a function of HL derived macrophages in matrix remodeling. Furthermore, we identified factors produced by HL cells that mediate the differentiation into this specific M2 type. Taken together our observations support a model in which HRS cells are able to induce monocyte differentiation into M2-related macrophages to rebuild the lymphoma microenvironment. Further molecular and functional studies are in progress to characterize mutual interactions between TAMs and HRS cells more deeply.

P.B2.04.02

Tks4 promotes tumor progression via modulation of the tumor-associated stroma

E. Boldizsár[1], *D. Laoui*[2], *A. Gyöngyösi*[1], *J. Van Ginderachter*[2], *Á. Lányi*[1];
[1]*University of Debrecen, Debrecen, Hungary,* [2]*Vrije Universiteit Brussel, Brussels, Belgium.*

Background: Tks4's (tyrosine kinase substrate with 4 SH3 domains) involvement in the regulation of cell migration, the development of functional podosomes and lamellipodia suggested that it plays a role in tumor progression. However, the function of Tks4 in the regulation of the tumor-associated stroma (TAS) - an essential, rate limiting factor of tumor growth and metastasis - is unknown. **Results:** Here we show that the murine melanoma B16-F10 and Lewis lung carcinoma (LLC) cells injected into Tks4-deficient mice develop significantly smaller tumors compared to those injected into wild-type animals. In addition, in bone marrow transfer experiments, animals that received Tks4-deficient bone marrow developed smaller tumors, strongly suggesting that the immune cells of TAS may be responsible for the observed differences. To identify which immunocyte subsets are affected by the absence of Tks4 LLC were injected into full-body $Tks4^{null}$ mice or mice in which Tks4 was eliminated in lineages expressing lysozyme M, affecting mainly macrophages and neutrophils. We found impaired tumor growth in Tks4-KO animals in both tumor models. Furthermore, the distribution of tumor-associated immune cells, surface marker expression, gene expression and the functional activity of the above cell populations was also influenced by Tks4. **Conclusion:** Tks4 expressed in myeloid lineages has a significant impact on the functional activity of TAS and appears to be a positive regulator of the tumor-associated immunosuppressive stroma. **Grants and fellowships**: Hungarian National Research, Development and Innovation Office (NKFIH, K-109444) (ÁL), EFIS-IL Short-term Fellowship (EB)

P.B2.04.03

The anti-tumor activity of brown adipose tissue: Function of the NLRP3 inflammasome and Caspase 1/11 of fat tissue in the modulation of breast cancer

L. C. Corrêa[1], *L. D. Dourado*[1], *R. A. Almeida*[1], *T. F. Furquim*[1], *A. M. Martins*[1], *M. E. Eberlin*[2], *K. M. Magalhães*[1];
[1]*University of Brasília, Brasília, Brazil,* [2]*University of Campinas, São Paulo, Brazil.*

Fat tissues can regulate cancer development by modulating inflammatory response. However, the role of NLRP3 inflammasome in white and brown adipose tissues in this context is poorly understood. Here, we aimed to characterize the role of caspase 1/11 and NLRP3 inflammasome components in the lipidomic profile of brown and white adipocytes and their function in breast cancer cells activation. Brown and white adipose tissue were isolated from wild type, caspase-1/11 and NLRP3 knockout mice. White and brown adipocytes tissues from these mice were analyzed by Electrospray Ionization Mass Spectrometry (ESI-MS). Conditioned medium from these adipocytes were used to stimulate breast cancer cells 4T1. Breast cancer cells lipid droplet biogenesis was analyzed by Bodipy or Oil Red staining followed by flow cytometry or Microscopy analysis, respectively. Breast cancer cells viability was assessed by MTT assay. Our data showed that white adipocytes conditioned medium triggered significant higher levels of lipid droplet biogenesis in breast cancer cells compared to brown adipocytes stimulation or unstimulated cells. The secretion product of brown adipose tissue decreased the viability of tumor cells. ESI-MS data showed that both fat tissues have distinct lipidic metabolites and the absence of inflammasome components changed significantly the profile of these fat cells. Taken together, our data showed white and brown adipocytes presented distinct lipidomic profile and the absence of NLRP3 inflammasome components may influence directly their lipidomic composition and their ability to activate breast cancer cells, suggesting an important mechanism that may be involved in their differential function in carcinogenesis. **Support**: CNPq

P.B2.04.04

Pro-tumoral role of complement activation in murine sarcoma models

S. Di Marco[1], *E. Magrini*[1], *C. Perucchini*[1], *K. Berthenet*[1], *M. Barbagallo*[1], *A. Ponzetta*[1,2], *C. Garlanda*[1,2], *A. Mantovani*[1,2];
[1]*Humanitas Clinical and Research Centre, Rozzano, Italy,* [2]*Humanitas university, Rozzano, Italy.*

Cancer related inflammation (CRI) plays a fundamental role in fuelling tumor appearance and development. Although the important contribution of complement activation to inflammation, its role in CRI still remains understudied. Recently our group demonstrated the pro-malignant role of complement activation in models of mesenchymal (3-MCA-induced) and epithelial (DMBA/TPA-induced) inflammation-driven skin carcinogenesis, showing that mice deficient for the key molecule C3 were protected from tumor development. First we observed the deposition of C3-cleavage products on vessels and tumor cells of sarcomas, while it was absent in normal tissues. C3 deposition on tumor cells was also observed *in vitro*, both on 3-MCA-derived sarcoma and on different murine cancer cell lines. Interestingly, both *in vitro* and *in vivo* experiments suggested that the activation of the classical and lectin pathways was involved in this process. Then, we investigated C3-downstream mechanism(s) of protection in two sarcoma murine models. We observed that $C3^{-/-}$ but not the $C5aR^{-/-}$ mice were protected from tumor growth in a transplantable model of sarcoma (MN-MCA), as well as in the 3-MCA-induced carcinogenesis model. $C3^{-/-}$ mice showed a protective phenotype which was associated with reduced macrophage and enhanced $CD8^{+}$ frequencies in tumor, suggesting that these cells could play a role in the protection. Similar results were obtained with $C3aR^{-/-}$ mice, suggesting that the C3a/C3aR axis was most likely responsible for the protection from tumor development observed in C3-deficient mice. All together our results indicate that complement activation occurs in tumor and contributes to sarcoma development.

P.B2.04.05

Leptin decreases susceptibility of breast cancer cells to NK-Lysis via PGC-1a pathway: Linking tumor progression with obesity

A. GATI[1,2]*, Hichem Bouguerra, Amal Gorrab, Stephan Clavel, Jean-François Louet, Guissouma Hager;*
[1]Faculté des Sciences de Tunis, EL manar, Tunisia, [2]Univeristy of Tunis El Manar, Faculty of science of Tunis, Tunisia.

Several studies established a link between obesity and breast cancer (BC) development. Yet, the mechanisms underlying this association are not understood. Among the diverse adipocytokine secreted by hypertrophic adipose tissue, leptin is emerging as a key candidate molecule linking obesity and cancer, since it promotes proliferation, migration and invasiveness of tumors. However, the potential implication of leptin on tumor escape mechanisms remains unknown. This study aims to explore the effect of leptin on tumor resistance to NK lysis and the underlying mechanism. We found that leptin promotes both BC resistance to NK92-mediated lysis and β oxidation on MCF-7, by the up-regulation of a master regulator of mitochondrial biogenesis, the Peroxisome proliferator activated receptor coactivator-1 α (PGC-1α). Using adenoviral approaches, we show that acute elevation of PGC-1α enhances the fatty acid oxidation pathway and decreases the susceptibly of BC cells to NK92-mediated lysis. Importantly, we identified new regulatory functions of PGC-1α and leptin in regulating the expression of the hypoxia inducible factor-1 alpha (HIF-1α) by tumor cells, a transcriptional factor with pleiotropic role in cancer. We further demonstrate that basal BC cells MDA-MB-231 and BT-20 exhibit an increased PGC-1α mRNA level, an enhanced activity of oxidative phosphorylation and are more resistance to NK92 lysis in comparison with luminal BC cells (MCF7 and MDA-361). Altogether, our results demonstrate for the first time how leptin could promote tumor resistance to immune attacks. Reagents blocking leptin or PGC-1α activity might aid in developing new therapeutic strategies to limit tumor development in obese BC patients.

P.B2.04.07

EZH1 was involved in inhibitory effect of miR-20a on the growth and metastasis of HCC

J. HUANG*, Q. Zhang, X. Deng, Y. Cai, X. Tang, X. He;*
Guangdong Provincial Key Laboratory of Organ Donation and Transplant Immunology, The First Affiliated Hospital, Sun Yat-sen University, GUANGZHOU, China.

Backgroud: Many miRNAs are associated with hepatocellular carcinoma (HCC). We found that miR-20a was down-regulated in HCC and could inhibit the proliferation and metastasis of HCC cells. It is worth to study deeply. **Objective:** To investigate the effect and mechanism of miR-20a on the growth and metastasis of HCC. **Methods:** transfection efficiency of miR-20a was detected by immunofluorescence and qRT-PCR; HCC cell proliferation was evaluated by CCK8 and plate clone formation; transwell experiments and cell scratch experiments were used to detect the invasion and migration of HCC cells; bioinformatics analysis and luciferase assay were used to search and verify miR-20a targets; tumorigenic ability and metastasis ability of SMMC7721 were observed by xenograft tumor growth assay in nude mice; the expressions of miR-20a and EZH1 were detected by qRT-PCR, in situ hybridization and immunohistochemistry; the levels of H3K27me, H3K27me2, H3K27me3 and PRC2-associated proteins were evaluated by immunohistochemistry. **Results:** miR-20a was down-regulated in HCC cell lines and inhibited HCC cell proliferation, invasion and migration. miR-20a could directly target EZH1, rather than EZH2. Overexpression of miR-20a could inhibit the growth and metastasis of SMMC7721 cells in vivo. Compared with adjacent tissues, miR-20a was decreased while EZH1 was increased in HCC tissues. H3K27me, H3K27me2 and H3K27me3 were up-regulated in HCC tissues. EZH2 and EZH1 were increased in HCC while SUZ12, EED and AEBP2 did not change significantly. **Conclusions:** EZH1 is the direct target of miR-20a and miR-20a/EZH1 axis is associated with growth and metastasis of HCC by modulating H3K27 methylation.

P.B2.04.08

Fratricide tumor-associated macrophages contribute to tumor destruction following chemotherapy

M. Laviron*, P. Loyher, P. Hamon, C. Combadière, A. Boissonnas;*
Centre d'Immunologie et des Maladies Infectieuses, Paris, France.

Tumor-associated macrophages (TAMs) are the most abundant cells within the tumor microenvironment and are thought to be implicated in tumor growth, metastasis formation and chemotherapy resistance. We recently highlighted the dual origin of TAMs in lung cancer in mice, being composed of both resident interstitial (Res-TAMs) and monocyte-derived (MoD-TAMs) macrophages. These subsets exhibited different sensitivity to the alkylating agent cyclophosphamide, as both were reduced in the early days following chemotherapy but MoD-TAMs transiently recovered after treatment while Res-TAMs did not. This MoD-TAMs wave was associated with tumor clearance but their level eventually dropped and tumor progression rebounded concomitantly. This observation unveils that TAMs' usefulness in chemotherapy efficacy is unclear. We thus attempted to decipher the relative contribution of these subsets in the first days following treatment. Using two-photon microscopy and histological analysis on mice expressing fluorescent reporters allowing for the discrimination between Res-TAMs and MoD-TAMs, we seek to characterize the distribution and dynamics of both populations during the first 3 days after treatment. Chemotherapy induced a rapid dissociation of the tight interactions between tumor cells, associated with an increased density of MoD TAMs, displaying higher protrusive activity between the tumor cells. In contrast, Res-TAMs in tumor nodules were rapidly eliminated following chemotherapy. Surprisingly, in mice deficient for the chemokine receptor CCR2, lacking MoD-TAMs, tumor destruction was not observed and Res-TAMs seemed to be less eliminated by cyclophosphamide. Our observations suggest an unexpected fratricide role for MoD-TAMs in the early phase following chemotherapy.
Support: Contrat doctoral de Sorbonne Université, ARC foundation

P.B2.04.09

CCL17 in intestinal inflammation and cancer

R. Metzger*, A. B. Krug;*
LMU Institut für Immunologie, Planegg-Martinsried, Germany.

Colorectal cancer (CRC) is one of the most common cancers and a major cause of mortality. Pro-inflammatory and anti-tumor immune responses play a critical role in colitis associated and sporadic CRC. CCL17, a chemokine of the C-C family with its receptor CCR4, is mainly expressed by the $CD11b^+$ subset of dendritic cells, promotes intestinal inflammation in two mouse models of colitis and its expression correlates with disease activity in ulcerative colitis patients. CCL17 enhances toll-like-receptor (TLR) mediated cytokine induction, promotes pro-inflammatory Th cell differentiation and inhibits Treg expansion during colitis. TLR4, 7/8 or 9 ligation led to a lower IL12p40 secretion in CCL17 deficient DCs compared to heterozygous controls. CCL17 may play a dual role in the development of CRC as a driver of inflammation and cancerogenesis as well as a modulator of anti-tumor immune responses. The role of CCL17 for intestinal tumor development was investigated using the murine colitis associated cancer model induced by AOM/DSS and the APC1638N mutant mouse model for sporadic CRC. In both models increased CCL17 expression was observed in the tumor tissue, indicating a role for this chemokine. By comparing tumor numbers in $CCL17^{eGFP/wt}$ heterozygous and $CCL17^{eGFP/eGFP}$ knockout mice, we found that CCL17 promoted tumor development in the colitis-induced (AOM/DSS) model, but had only a minor effect on spontaneous intestinal cancerogenesis. Preliminary data obtained from syngeneic subcutaneous tumor models show that CCL17 expression is also induced in these tumors and drives tumor growth, indicating that CCL17 shapes the tumor microenvironment independently of intestinal inflammation.

P.B2.04.10

Tumor cells secretome impairs the plasmacytoid dendritic cells compartment in metastatic melanomas

M. Monti[1]*, R. Vescovi[1], D. Moratto[2], F. Consoli[3], M. Rossato[4], L. Paolini[1], L. Benerini Gatta[1], M. Bugatti[1], V. Salvi[1], D. Bosisio[1], M. Maio[5], E. Fonsatti[5], M. Simbolo[6], A. Scarpa[6], M. Delledonne[4], P. Bergese[1], S. Sozzani[1], F. Facchetti[1], W. Vermi[1,7];*
[1]University of Brescia, Brescia, Italy, [2]"Angelo Nocivelli" Institute, ASST Spedali Civili of Brescia, Brescia, Italy, [3]ASST Spedali Civili of Brescia, Brescia, Italy, [4]University of Verona, Verona, Italy, [5]University Hospital of Siena, Siena, Italy, [6]ARC-Net Research Centre, University of Verona, Verona, Italy, [7]Washington University School of Medicine, Saint Louis, United States.

Introduction: Among immune cells involved in cancer immunity, Plasmacytoid Dendritic Cells (PDC) exert an important role bridging the innate and adaptive immune responses. Properly activated PDC can eliminate cancer cells through the Toll-like receptor (TLR) 7 and TLR9-dependent type I Interferon (I-IFN) production and other effector functions. PDC infiltration has been documented in several types of cancer, however, their role within the melanoma immune-contexture is still largely unknown.
Methods: PDC frequency was evaluated in a cohort of 29 Metastatic Melanoma (MM) patients. Intracellular IFN-α and IP-10 were measured on Peripheral Blood Mononuclear Cells (PBMC) after TLR7/9 agonists administration. Purified PDC were exposed to melanoma cell line supernatants (SN-mel). *In vitro* viability and production of IFN-α and IP-10 were evaluated by flow cytometry. Finally, the mRNA-Sequencing (mRNA-Seq) analysis was performed.
Results: Circulating PDC are severely reduced and defective in IFN-α and IP-10 production in MM patients. Furthermore, *in vitro* exposure to SN-mel resulted in significant PDC death. Also, SN-mel induced a significant decrease of IFN-α and IP-10 production by PDC. Finally, mRNA-Seq has revealed deregulation of I-IFN pathway by different SN-mel exposure.
Conclusions: In this study we demonstrate that in MM the circulating PDC compartment is collapsed. In addition, the residual PDC component is not functional. Furthermore, our *in vitro* findings indicate that components released by melanoma cells have a relevant effect on the survival and anti-tumor activity of fully differentiated PDC. The underlying molecular mechanisms identification is underway through the mRNA-Seq data analysis.
This work was supported by AIRC (IG-15378).

P.B2.04.11

Survival and stemness of HT29 colon cancer cells is influenced by self-DNA via crosstalk of TLR9- and autophagy signaling

G. Müzes[1], *A. L. Kiss*[2], *F. Sipos*[1];
[1]*2nd Department of Medicine, Budapest, Hungary,* [2]*Semmelweis University, Budapest, Hungary.*

TLR9 and autophagy pathways seem to be bi-directionally involved in carcinogenesis. In cancer cells the biological consequences of the TLR9 and autophagy crosstalking induced by self-DNA is poorly documented. HT29 cells were incubated with genomic(g), artificially hypermethylated(m), fragmented(f), and hypermethylated/fragmented(m/f) self-DNA sequences. Cell viability, induction of apoptosis, cell proliferation, transcriptional alterations of TLR9-signaling and the autophagy process were assayed, respectively. Moreover, autophagy proteins, morphologic features of apoptosis and autophagy, and the presence of colonospheres were examined. Following incubation with g-, m-, and m/f-DNAs viability and proliferation of HT29 cells decreased and percentage of apoptotic cells increased, while f-DNA resulted in an appreciably increase of cell survival. Methylation of self-DNA resulted in decrease of TLR9 expression, but did not influence the positive effect of DNA fragmentation on overexpression of Myd88 and TRAF6, and downregulation of TNFα. Fragmentation of DNA abrogated the effect of methylation on IRAK2, NFκB and IL-8 mRNA upregulation. G- and f-DNAs significantly upregulated Beclin1, Atg16L1, and LC3 autophagy genes. On a protein level, the results were parallel with the gene expressions. According to TEM, presence of autophagy was observed in each groups. Incubation with m-DNA activated mitophagy, and suppressed tumor cell survival by inducing features of apoptosis. F-DNA treatment enhanced cell survival, activated macroautophagy and lipophagy. As a marker of stemness, CD133+ colonospheres were present only after m-DNA incubation. Our data provide evidence for an interplay between TLR9- and autophagy signaling using HT29 cells subjected to modified self-DNA with remarkable influences on survival and stemness of cancer cells.

P.B2.04.12

IL17a, IL21 and IL22 serum levels in colorectal cancer

N. İbrahimli[1], ***F. Ozmen***[1], *C. E. Guldogan*[2], *M. M. Ozmen*[2];
[1]*Department of Basic Oncology, Cancer Institute, Hacettepe University, Ankara, Turkey,* [2]*Department of Surgery, Medical School, Istinye University, Istanbul, Turkey.*

Background and Aim:T-helper 17 (Th17) pathway plays an important role in promoting colorectal cancer. The aim of this study was to evaluate serum levels of pivotal cytokines (IL-17a, IL-21 and IL-22) and their correlation with clinicopathologic parameters of colorectal cancer. **Patients and Methods:**40(19F) patients with colorectal cancer with a median(range) age of 61(30-83)years and 40(18F) healthy controls with no history of any cancer with a median(range) age of 44(25-58) years were included in the study. Preoperative blood samples were collected from the patients.Correlation between serum interleukin levels and stage, differentiation, presence of metastasis, lymph node invasion, perineural and vascular invasions were also evaluated.**Results:**IL-17, Il-21 and IL-22 levels as pcg/ml were 3.11(2.99-3.73), 108.3(11.9-1394), 38.8(38.4-42.9) respectively in patients with colonic cancer. Whereas, they were 1.3(0.7-6.2); 123.12(61.8-1157.6); 21.3(0.15-143.9) pg/ml respectively in healthy controls. IL-17 and Il-22 were found to be increased significantly in patients with colonic cancer (p<0.001). Cut-off value for the significance of IL-17 was found to be 2.755 pg/ml and cut-off value for IL-22 was found to be 35.63 pg/ml. Any values over those were found to be correlated with colonic cancer(*p<0.001*). On the other hand, presence of metastatic lymph nodes, vascular invasion and perineural invasion were all found to be correlated with increased IL-17 and IL-22 levels(*p<0.001*). Patients with distant metastasis(n=5) also had significantly increased levels of IL-17 and IL-22. **Conclusions:**There was a strong correlation between increased levels of IL-17 and IL-22 not only with the presence of cancer but also with the presence of invasion and metastasis.

P.B2.04.13

The sweet side of pancreatic cancer: the tumor glyco-code contribute to the tolerogenic microenvironment.

E. Rodrguez[1], *S. Schetters*[1], *G. Kazemier*[2], *E. Giovannetti*[3], *J. Garcia-Vallejo*[1], *Y. van Kooyk*[1];
[1]*Department of Molecular Cell Biology and Immunology, VUmc., Amsterdam, Netherlands,* [2]*Department of Surgery, VUmc., Amsterdam, Netherlands,* [3]*Department of Medical Oncology, CCA, VUmc., Amsterdam, Netherlands.*

Pancreatic cancer, one of the most aggressive malignancies, is characterized by an immune-suppressive tumor microenvironment. It has been postulated that changes in the glycosylation of tumor cells, which we call tumor glyco-code, have an impact in the induction of a tolerogenic microenvironment. Immune cells express different glycan-binding receptors (GBR) that can sense and respond to changes in the glyco-code; which often leads to inhibitory immune processes. In this work, we characterize changes in glycosylation during pancreatic cancer progression and analyze how they interact with the immune system.
An extensive glycoprofiling analysis in 10 different pancreatic cancer cell lines, which revealed that fucosylated antigens (eg. Lewis x, Lewis y, VIM-2) are expressed in cells that markers an epithelial phenotype, while they are absent in mesenchymal-like cells. The presence of those structures is associated with the expression the enzyme GALNT3, which expression is regulated by ZEB1 during epithelial to mesenchymal transition.
The presence of fucose-containing glycans in epithelial cells was correlated with a high DC-SIGN binding, a lectin receptor with well described immuno-modulatory properties. Interestingly, tumor cell lines are capable to induce the differentiation of monocytes towards DC-SIGN+ tumor associated macrophages (TAMs), characterized by the expression of CD163, CD14 and the mannose receptor. This data suggests that tumor-induced DC-SIGN+ TAMs cells could be differentially modulated by epithelial or mesenchymal cells. DC-SIGN signalling in TAMs may be a characteristic in their interaction with epithelial cells and therefore contribute to early local tolerance in the primary tumor.

P.B2.04.14

Refractory activation state of CD29 in multiple myeloma plasma cells from extramedullary sites as a possible new mechanism to explain their disseminated behaviour.

A. Roncancio-Clavijo, *E. Martínez-Viñambres, L. Villar, E. Roldán;*
Hospital Universitario Ramón y Cajal, Madrid, Spain.

Multiple myeloma (MM) is a disease characterized by malignant proliferation of clonal plasma cells (PC), usually restricted to the bone marrow (BM). However, in a subset of cases clonal PC expansion can occur outside of the BM and can present as plasma cell leukemia (PCL) or extramedullary MM (EMM). The mechanisms that explain PC egress out of the BM are poorly understood. Here we report that, in contrast to MM cases without disseminated disease, malignant PC in blood or extramedullary sites did not express the high-affinity form of CD29 and were refractory to cation-induced CD29 activation. Patients and methods Six MM patients with EMM or PCL and 15 with disease confined to the BM were studied. BM aspirates, peripheral blood or pleural effusions were stained with conjugated mAb against CD38, CD138, CD29 (clone MAR4 or HUTS21, detecting constitutive or active epitopes, respectively). For HUTS21 regulation experiments, tumoral cells were incubated with different Mn^{2+} concentrations (0.01-10mM). Antigen expression was monitored by flow cytometry. Results PC from EMM or PCL patients displayed very low levels of HUTS21 active epitope (median: 1.8%±1.6%), in contrast to PC from MM patients with disease confined to the BM (median: 58%±17%; p<0.001). Moreover, clonal PC in pleural fluids or blood showed a very poor response to exogenously added Mn^{2+}, even at 10mM concentration (median: 2.6%±2.2%) in comparison to PC from patients without disseminated disease (median: 93%±5%; p<0.001). Conclusion Malignant PC from EMM or PCL patients do not express active CD29 since were unresponsive to regulatory factors.

P.B2.04.15

The p38 MAPK pathway influence of dendritic cells treated with tumor-derived soluble factors

A. S. A. Santos, *E. M. Araujo, A. M. Vale;*
Federal University of Maranhão, São Luis, Brazil.

Introduction: Dendritic cells are antigen-presenting cells responsible for initiating adaptive immune response or induce peripheral tolerance. The microenvironment can induced change in DCs functional status. Thus, in tumor microenvironment, the suppressor factors cause tolerogenic phenotype in DCs that contribute to the development and progression of cancer. The aim was evaluated the DCs differentiation from human monocytes (Mo-DCs) in presence tumor-derived soluble factors, as well as a possible signaling pathway involved in this process. Materials and Methods: Mo-DCs were obtained from monocyte culture with GM-CSF and IL-4 and TNF as maturation stimulus. During the differentiation process, DCs were treated with p38 MAPK pathway inhibitor and 30% (v/v) supernatant of tumor cells MCF-7 and analyzed morphologically and phenotypically. Results: The results showed that the protocol used was efficient for the generation of DCs in vitro. Supernatant did not alter tumor HLA-DR expression, however, reduced the expression of costimulatory molecule CD86. The combined treatment with tumor supernatant and p38 pathway inhibitor promoted an increase in HLA-DR expression, indicating that p38 pathway may be involved in DC differentiation process. However, DCs treated tumor supernatant had less CD86 expression continued with this profile even with the use of a p38 inhibitor, indicating that the p38 pathway may not be the only one involved in this process. Conclusions: Thus, the results suggest that the supernatant of tumor cells alters the functional characteristics of DCs, acting in the maturation process, and that this influence of the supernatant can be dependent upon, but not exclusively, the p38 pathway.

P.B2.04.16
'Bystander' effects of senescent tumour cells induced by docetaxel or IFNγ+TNFα

***O. Sapega**, M. Romana, M. Blanka, H. Zdeněk, R. Milan;*
Institute of Molecular Genetics of the ASCR, v. v. i., Prague, Czech Republic.

Cellular senescence is a process of a cell-cycle arrest in response to various inducers. Senescence is characterized by a series of morphological changes and is typically connected with secretion of bioactive molecules (senescence-associated secretory phenotype, SASP). Thus, senescent cells can affect their local environment and through their SASP induce 'bystander' senescence. To investigate a relationship between the phenotype of senescent cells and "bystander" senescence induction, we induced senescence by treatment of tumor cells (TC-1 and B16) with two different inducers - cytotoxic agent docetaxel (DTX), and by the combination of cytokines IFNγ+TNFα. We have demonstrated that DTX induced senescence both in the TC-1 and B16 tumor cell lines, which was proved by *in vitro* and *in vivo* growth arrest, detection of increased p21 expression, positive beta-galactosidase staining, and the typical SASP capable to induce 'bystander' senescence. On the other hand, treatment with the combination of IFNγ+TNFα induced a proliferation arrest only in B16 cells. Despite the presence of some characteristic features resembling senescent cells (proliferation arrest, morphological changes), B16 senescent cells were able to form tumors *in vivo* and started to proliferate upon the cytokine withdrawal. In addition, B16 cells were not able to induce 'bystander' senescence. Collectively, our work presented here has established a murine model that is beneficial for research of direct and 'bystander' biological effects of senescent cells. This work was supported by the research grant No. 15-24769S provided by the Czech Science Foundation.

P.B2.04.17
Platelet-activating factor (PAF) receptor promotes tumor cell repopulation after radiotherapy

***I. A. Silva-Junior**[1], A. P. Lepique[2], S. Jancar[2];*
[1]Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil, [2]University of São Paulo, São Paulo, Brazil.

Radiotherapy generates oxidized phospholipids that activate platelet-activating factor receptor (PAFR) associated with pro-tumorigenic effects. Here, we investigated the involvement of PAFR in tumor cell survival after irradiation. Cervical cancer samples presented higher levels of PAF-receptor gene (PTAFR) when compared with normal cervical tissue. In cervical cancer patients submitted to radiotherapy (RT), the expression of PTAFR was significantly increased. Cervical cancer-derived cell lines (C33, SiHa, HeLa, TC-1) and squamous carcinoma cell lines (SCC90 and SCC78) express higher levels of PAFR mRNA and protein than keratinocytes. Gamma radiation increased PAFR expression and induced PAFR ligands and prostaglandin E2 in these tumor cells. Irradiated, dying TC-1 feeder cells increased the proliferation of living TC-1 reporter cells in a PAFR-dependent way. The blocking of PAFR with the antagonist CV3938 before irradiation increased tumor cells death. Similarly, human carcinoma cells that do not express PAFR (KBM) were irradiated and co-injected with cells transfected with PAFR gene (KBP) into RAG mice. These tumors grew significantly bigger than the ones formed by co-injection of live KBM with irradiated KBM. Tumor repopulation correlated with increased M2 (CD206+) macrophages in KBP tumors. Taken together, these results suggest that PAFR ligands generated during tumor irradiation activates PAFR in tumor cells and enhance their survival and proliferation while the activating of PAFR in tumor macrophages reprogramming them to a pro-tumor profile. This may be relevant in tumor repopulation phenomenon after radiotherapy. We propose that PAF receptor represents a possible target for improving the efficacy of radiotherapy through inhibition of tumor repopulation.

P.B2.04.18
Oncostatin M promotes tumor progression in skin squamous cell carcinoma

***M. Simonneau**[1], E. Frouin[1,2], C. Jermidi[1,2], J. F. Jegou[1], A. Barra[1,2], I. Paris[1], V. Huguier[1,2], P. Levillain[2], S. Cordier-Dirikoc[3], N. Pedretti[3], F. X. Bernard[1,3], J. C. Lecron[1,2], F. Morel[1], L. Favot[1];*
[1]LITEC, Poitiers, France, [2]CHU de Poitiers, Poitiers, France, [3]BioAlternatives, Gençay, France.

Cutaneous squamous cell carcinoma (cSCC) is one of the most frequent keratinocyte malignancies worldwide and is chemotherapy resistant. Surgery is the curative treatment but there isn't any alternative in advanced cSCC. Reprogramming tumor microenvironment and tumor immunosuppressive mechanisms is a new therapeutic approach. Indeed, depending on cytokine expressed in tumor microenvironment, immune cells can inhibit (Th1/M1 cells) or enhance (Th2/M2 cells) tumor development. It was previously showed that Onconstatin M (OSM) had pleiotropic effects on cancer cells. OSM can promote cancer by inducing tumor cells motility, invasiveness or by reprogramming immune cells toward a more permissive phenotype (M2 polarization). Our previous data showed that OSM has proinflammatory effects on skin and modulate normal keratinocyte phenotype both *in vitro* and *in vivo*.
In this study, we hypothesized that OSM could be involved in cSCC development.
We showed that OSM was overexpressed in human cSCC as well as other cytokines such as IL-6, IL-1β, IFNγ whereas IL-4 was decreased, suggesting a Th1/M1 polarization of cSCC microenvironment. *In vitro*, OSM induced STAT-3 and ERK signalization, modified gene expression, promoted proliferation and migration of malignant keratinocyte PDVC57 cells. PDVC57 cells grafted in skin mice led to cSCC development associated to OSM overexpression by immune infiltrated cells. Finally, we showed that the absence of OSM led to a 30% reduction of tumor size and reduced M2 polarization in tumor microenvironment. Collectively, these results support a pro-tumoral role of OSM in cSCC development and suggest a new therapeutic approach targeting this cytokine.

P.B2.04.19
Immune cell composition in non-small cell lung cancer

***B. Stankovic**, H. Korsmo Bjørhovde, R. Skarshaug, H. Aamodt, E. Müller, C. Hammarström, K. Beraki, E. Bækkevold, P. Woldbæk, Å. Helland, O. Brustugun, I. Øynebråten, A. Corthay;*
Oslo University Hospital, Oslo, Norway.

Non-small cell lung cancer (NSCLC) has highest mortality rates in the world. Development of immunotherapy, in particular immune checkpoint inhibitors, has improved survival, but only for a fraction of patients. This could be due to variation in the immune response to cancer between individual patients, and analysis of tumor-associated immune cells could prove valuable in the selection of clinical treatment. However, the exact immune cell composition in NSCLC remains unclear. Here, we used flow cytometry to analyze immune cell composition in NSCLC of 69 patients. T cells dominated the lung cancer landscape (on average 46.1% of all CD45+ cells). Among these CD4+ T cells were the most abundant (25.8%), closely followed by CD8+ T cells (20.6%). CD19+ B cells were the second most common population (on average 15.9%) including naive, germinal center, memory and plasma cell subsets. Macrophages and natural killer (NK) were less abundant, composing 5.4% and 4.3% respectively. Dendritic cell (DC) populations, including plasmacytoid DCs, CD1c+ DCs and CD141+ DCs, together represented 1.9% of all immune cells. Among granulocytes, the neutrophils were variably present (0-35%), basophils (0.5%) and eosinophils (0.6%) were rare, while mast cells constituted 1.5%. Analysis of immune cell composition in 69 tumors revealed individual differences between the patients. Interestingly, across the cohort of patients, only B cells showed a significantly higher representation in the tumors compared to the distal lung, while macrophages and NK cells had a relatively lower representation. This shows that the immune cell repertoire is significantly influenced by the tumor presence.

P.B2.04.20
Characterization of tumor-induced immune suppression by monocytic myeloid-derived suppressor cells

***G. F. van Wigcheren**, G. Flórez-Grau, M. Tazzari, J. M. de Vries, C. G. Figdor;*
Radboud Institute for Molecular Life Sciences, Radboudumc, Nijmegen, Netherlands.

Monocytic myeloid-derived suppressor cells (M-MDSCs) are major regulators of immune responses in cancer as greatly evidenced in melanoma patients. Clinical studies have shown that circulating MDSC levels are closely related to tumor stage and prognosis. This tumor-induced conversion of monocytes into M-MDSCs promotes cancer progression by dampening spontaneous and therapeutic immune responses. A better understanding of M-MDSCs conversion and suppressive function is needed to identify potential targets for drugs that can inhibit cancer immune evasion and contribute to successful application of cancer immunotherapy.
M-MDSCs were generated out healthy donor monocytes and compared to monocyte-derived dendritic cells (MoDCs) and tolerogenic MoDCs (TolDCs) from the same donor. Phenotypic characterization of myeloid maturation and activation markers was performed using multicolor flow cytometry. Suppressive activities of generated monocytic subsets were compared in co-culture assays measuring the activation of T cells.
The culture protocol induced M-MDSCs with an immature monocytic morphology. These cells express myeloid lineage markers CD11b, CD33 and CD14 while downregulating HLA-DR expression. The expression of co-stimulatory markers CD86, CD83 and CD80 remained low. M-MDSCs were more potent in inhibiting T cell activation in co-culture assays than TolDCs. The conversion of healthy monocytes into pathologically activated M-MDSCs as seen in melanoma patients can be recapitulated using an ex vivo culture protocol. Ex vivo generated M-MDSCs are characterized by a $CD11b^{+}CD33^{+}CD14^{+}HLA\text{-}DR^{low}$ phenotype and an immature morphology. These cells were potently able to suppress activated T cells as demonstrated in co-culture assays. Molecular studies are warranted into the MDSC conversion and their suppressive activity.

P.B2.05 Environmental regulation anti-tumor responses - Part 5

P.B2.05.01

Myeloid derived suppressor cells in smokers and in chronic obstructive pulmonary disease patients with and without lung cancer

V. Andreu[1,2], *V. Cunill*[1,2], *J. Verdú*[3,4], *C. Capó-Serra*[1,4], *M. I. Luna*[5,4], *A. Iglesias*[4], *A. Alonso*[3,4], *B. G. Cosío*[5,4], *J. M. Ferrer*[1,4], *J. Sauleda*[3,4], *J. Pons*[1,4];
[1]*Immunoloy department. Hospital Universitari Son Espases, Palma De Mallorca, Islas Baleares, Spain, Palma, Spain,* [2]*Institut d'Investigació Illes Balears (IdISBa), Palma, Spain,* [3]*Neumology department. Hospital Universitari Son Espases, Mallorca, Balearic Islands, Spain, Palma, Spain,* [4]*Institut d'Investigació Illes Balears (IdISBa), Mallorca, Balearic Islands, Spain, Palma, Spain,* [5]*Neumology department. Hospital Universitari Son Espases, Palma De Mallorca, Islas Baleares, Spain, Palma, Spain.*

Introduction: Chronic obstructive pulmonary disease (COPD) and lung cancer (LC) are prevalent diseases causing morbidity and mortality worldwide. Tobacco smoking is the main risk factor for both diseases. However, not all smokers develop COPD or LC. Several inflammatory cells have been involved in both diseases and can favor the chronic inflammatory process and/or promote carcinogenesis. Myeloid derived suppressor cells (MDSC) contribute to maintain the anti-cancer and the homeostasis of the inflammatory process.
Objective: We aimed to evaluate MDSC subpopulation in COPD and LC patients.
Materials and methods: Percentages of MDSC ($Lin2^-DR^-CD11b^+CD33^+$) were evaluated by flow cytometry in peripheral blood samples from COPD and smokers. In both groups, patients with and without LC were also separately evaluated.
Results: Although we did not find differences between COPD patients and smokers without COPD, we observed a trend toward higher percentages in the latter group (3,83±0,83 and 5,03±2,00% respectively; p=0,52). Percentages were higher in COPD patients with LC compared to COPD patients without LC (4.26±0.92 and 1,02±0,58% respectively; p=0,19) however the differences did not reach statistically significance again. Similarly, there was a trend toward higher percentages of MDSC in smokers with LC compared to smokers without LC (6,72±3,31 and 2,6±1,06% respectively; p=0,34).
Conclusion: We have found a trend toward higher percentages of MDSC in smokers without COPD when compare to COPD patients. When we evaluated LC patients in COPD and in smokers without COPD there was a trend toward higher percentages of MDSC in LC patients from both groups.

P.B2.05.02

Evaluation of soluble HLA-G and plasma cytokines in papillary thyroid carcinoma patients

B. C. Bertol[1], *J. N. de Araújo*[2], *I. Sadissou*[1], *P. Sonon*[1], *F. C. Dias*[1], *R. H. Bortolin*[2], *N. L. de Figueiredo-Feitosa*[1], *L. C. de Freitas*[1], *S. R. Tarrapp*[3], *C. C. Ramos*[3], *A. D. Luchessi*[2], *J. C. Freitas*[2], *L. M. Maciel*[1], *V. N. Silbiger*[2], *E. A. Donadi*[1];
[1]*University of São Paulo, Ribeirão Preto, Brazil,* [2]*Federal University of Rio Grande do Norte, Natal, Brazil,* [3]*Hospital Liga Norte Riograndense Contra o Câncer, Natal, Brazil.*

Introduction: Considering that inflammation is a major component of papillary thyroid carcinoma (PTC) pathogenesis, we investigated the role of the immune checkpoint soluble HLA-G (sHLA-G) molecule and a panel of plasma cytokines in PTC patients. Materials and Methods: We studied 85 PTC patients before thyroidectomy and 80 healthy controls. Plasma levels of sHLA-G and 13 different cytokines (IL-1α, IL-1β, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17A, IFN-α, IFN-γ, TGF-β1 and TNF) were determined by ELISA and cytometry, respectively. Data were evaluated using univariate and multiple logistic regression analyses, and ROC curves. Results: Compared to controls, IL-6 levels were increased, while IL-1β, IFN-α and TGF-β1 levels were decreased in PTC patients. IFN-α and TGF-β1 efficiently discriminated patients from controls after multiple logistic regression analysis, in which IFN-α presented the best diagnostic performance. Increased IL-1β and decreased IL-12p70 levels were independently associated with larger tumors (>2.0 cm), while decreased sHLA-G levels were associated with local invasion of the tumor. PTC patients exhibiting poor therapy response presented higher levels of IL-5 and IFN-α. Conclusions: The decreased sHLA-G plasma levels observed in patients do not reflect the increased expression of HLA-G reported for PTC samples. The IFN-α and TGF-β1 levels were able to discriminate patients from controls. Additionally, pro-inflammatory and anti-inflammatory cytokines were associated with poor prognosis factors of PTC and poor response to therapy. Financial support: São Paulo State Research Foundation (FAPESP-grant #2015/26556-0), Federal Brazilian Research Foundations (CNPq-grant #304931/2014-1) and CAPES (CAPES/PROCAD-grant #88881-068436/2014-01).

P.B2.05.03

Investigation of the role of IL-36 cytokines in colon cancer

E. Brint, *A. Houston, C. O'Donnell;*
University College Cork, Cork, Ireland.

The IL-36 cytokines (IL-36α, IL-36β and IL-36γ) are a recently described subset of the interleukin-1 (IL-1) family of cytokines. Given the involvement of other IL-1 family members in the tumorigenic process, it is highly likely that these novel IL-36 cytokines also play a role in cancer. Here we show that IL-36α and IL-36γ is increased in human colorectal cancer tissue compared to adjacent non-tumour tissue, at both the mRNA and protein, whilst IL-36β is altered at the mRNA level only. Expression did not, however, correlate with stage, grade or patient prognosis. Transcription of the IL-36 receptor (IL-36R) was unchanged, with both tumour cells and immune cells in the tumour microenvironment expressing the receptor. Whilst no IL-36 cytokine altered colon cancer cell migration or invasion, IL-36γ strongly increased cellular proliferation in two colon cancer cell lines. IL-36α and IL-36γ also induced high levels of expression of CXCL-1, CCL-2, CCL-20 and IL-8 in colon cancer cells. Finally, expression of all IL-36 cytokines is strongly induced in these cells in response to the colonic tumour-promoting stimulus PGE_2. Taken together, these data show that certain IL-36 cytokines are increased in colon cancer and that tumour cells may respond to IL-36R stimulation in terms of a positive increase in proliferation and an induction of pro-tumorigenic chemokines.

P.B2.05.04

Cross-presentation of tumor-associated antigens by lung DC1 is lost in tumors by downregulation of TIM4 mediated efferocytosis

N. Caronni[1], *F. Simoncello*[1], *G. M. Piperno*[1], *K. E. Cervantes Luevano*[1], *S. Vodret*[1], *S. Bicciato*[2], *F. Benvenuti*[1];
[1]*Icgeb, Trieste, Italy,* [2]*Center for Genome Research Dept. of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy.*

Batf3-dependent DC1 are critical for the initiation of anti-tumor immunity and their abundance in tumors correlates with good prognosis and responses to immunotherapies. Yet, original DC1 functions are hit in the tumor microenvironment facilitating tumor escape. We addressed the mechanism underlying DC1 suppression in an orthotopic model of non-small-cell-lung-cancer (NSCLC). DC1 effectively cross-presented tumor antigens at early stages whereas this activity was lost in established tumors. DC1 isolated from tumor bearing lungs and fed with antigen ex-vivo confirmed a selective loss in formation of MHC class-I immune complexes from ingested antigens. Gene expression profiling showed modulation of transcripts involved in antigen processing/intracellular trafficking and exhaustion of inflammatory pathways. Notably, tumor associated DC1 strongly down-regulated Tim4 a receptor for phosphatidylserine implicated in apoptotic cell uptake (efferocytosis) in macrophages. We found that TIM-4 expression is restricted to DC1 among lung phagocytes and we confirmed loss of receptor expression by flow cytometry in tumor associated DC1. Notably, the uptake of dying cells and the cross-presentation of cell-associated antigens was significantly diminished in tumors. In addition, TIM-4 blockade inhibited efferocytosis by DC1 and cross-priming of tumor specific T cells. These data identify TIM4-mediated engulfment of dying cancer cells by DC1 as a lung specific mechanism of immune surveillance that is lost upon tumor progression.

P.B2.05.05

Modulation of Glucocorticoid Induced Tumor Necrosis Factor Receptor (GITR)-GITR Ligand (GITRL) interaction in Breast Cancer Cells under the control of Ataxia Telangiectasia Mutated (ATM) Promoter

B. Dayanc[1], *D. Yoyen Ermis*[2], *E. Dayanc*[3], *G. Esendagli*[2];
[1]*Izmir Biomedicine and Genome Center, Izmir, Turkey,* [2]*Hacettepe University Cancer Institute, Ankara, Turkey,* [3]*Izmir University of Economics, Faculty of Medicine, Izmir, Turkey.*

Despite treatment, basal-like breast cancers (BLBC) has poor prognosis and high mortality. We investigated ATM activity in BLBC cell lines with radiation and examined the viability of BLBC cells during the GITR-GITRL interaction. ATM expression levels in basal-like (MDA-MB-231, HCC38, MDA-MB-468) and luminal (MCF-7, BT-474, SK-BR-3) breast cancer cell lines were a found similar. We observed an increase in ATM (S1981) phosphorylation in these cells. HCC38 cells transfected with "pATM-GL3" Luciferase reporter plasmid showed high basal and post-radiation ATM activity with experiments suggesting a post-transcriptional control mechanism. When we investigated GITR and GITRL expressions in BLBC cells, we observed no change in expression levels with radiation. While MDA-MB-231 and MDA-MB-468 cell lines show high GITRL expression, HCC38 cell line was GITR positive. GITR+ HCC38 cells were incubated with recombinant GITRL protein at different serum concentrations (1% and 10%) and the change of cancer cells' viability, proliferation and amount of metabolically active viable cells were investigated with DRAQ7 staining, CFSE assay and MTT assay, respectively. Even though GITR stimulation only has not changed viability and proliferation of HCC38 cells, both ionizing radiation and GITR stimulation had a cumulative effect on cell viability. When cell death was assayed, a significant decrease in viability of the cells was observed, with simultaneously exposure to 80 ng/ml rGITRL and 5 Gy ionizing radiation. This study demonstrated that cumulative effect of GITR stimulation and ionizing radiation may affect the viability of breast cancer cells.

P.B2.05.06

Glioblastoma exploits glycosylation-mediated immune regulatory circuits for immune escape

S. A. Dusoswa[1], *J. Verhoeff*[1], *E. R. Abels*[2], *E. C. Rodriguez*[1], *S. J. Van Vliet*[1], *D. P. Noske*[1], *T. Würdinger*[1], *X. O. Breakefield*[2], *M. L. Broekman*[2,3], *Y. Van Kooyk*[1], *J. J. Garcia-Vallejo*[1];
[1]*VU University Medical Center, Amsterdam, Netherlands,* [2]*Massachusetts General Hospital, Harvard Medical School, Boston, United States,* [3]*University Medical Center Utrecht, Utrecht, Netherlands.*

Glioblastoma (GBM) is the most aggressive brain malignancy. Its histopathology is characterized by a significant infiltration with tumor associated macrophages and microglia (TAM), often comprising more than 30% of the tumor mass. TAMs express the macrophage galactose lectin (MGL) receptor in several tumor types, where it is thought to contribute to immune suppression upon binding to truncated O-linked glycans. Here we aim to elucidate the role of truncated O-glycans in GBM immune escape. To this end, GBM and control surgical samples were collected, and expression of MGL and its ligands was measured by flow cytometry, immunofluorescence, and ELISA. We detected significantly higher levels of the MGL receptor, and MGL-ligands in patient-derived GBM samples as compared to control samples. We then investigated the *in vivo* effects of varying levels of MGL ligands on immune composition in the tumor as well as systemically using an orthotopic immunocompetent GBM mouse model. Our data shows increased expression of MGL within several subpopulations of intratumoral myeloid cells as compared to mock injected mice. Our high dimensional mass cytometry analysis of tumors overexpressing truncated O-linked glycans revealed significantly increased subpopulations of immune suppressive TAMs. Our results suggest that GBM overexpress truncated O-linked glycans, and exploits glycosylation-mediated immune regulatory circuits for immune escape via immune suppressive TAMs. We hypothesize that manipulation of the MGL-MGL-ligand axis may provide new therapeutic avenues in preventing GBM immune escape.

P.B2.05.07

IDO dependent attenuation of NK cells contributes to enhanced mammary tumor growth in diabetic mice

N. M. Gajovic[1], *M. Jurisevic*[2], *N. Arsenijevic*[1], *M. Lukic*[1], *I. Jovanovic*[1];
[1]*Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia,* [2]*Department of Pharmacy, Faculty of Medical Sciences, University of Kragujevac, Serbia, Kragujevac, Serbia.*

Diabetic patients have higher incidence and mortality of cancer. Recent study revealed that hyperglycemia-induced oxidative stress is involved in the acceleration of tumor metastasis. We used model of high dose streptozotocin-induced diabetes to investigate its effect on tumor growth and modulation of antitumor immune response of 4T1 murine breast cancer in BALB/c mice. Diabetes accelerated tumor appearance, growth and weight, which was associated with decreased NK cells cytotoxicity against 4T1 tumor cells *in vitro*. Diabetes reduced frequencies of systemic NKG2D$^+$, perforin$^+$, granzyme$^+$, IFN-γ$^+$ and IL-17$^+$ NK cells, while increased level of PD-1 expression and production of IL-10 in NK cells. Diabetes decreased percentage of NKG2D$^+$NK cells and increased percentage of PD-1$^+$ NK cells also in primary tumor. Diabetes increased accumulation of IL-10$^+$ Tregs and TGF-β$^+$ myeloid derived suppressor cells (MDSCs) in spleen and tumor. Diabetic sera *in vitro* significantly increased percentage of KLRG-1$^+$ and PD-1$^+$ NK cells, decreased percentage of IFN-γ$^+$NK cells, expression of NKp46 and production of perforin, granzyme, CD107a and IL-17 per NK cell in comparison to glucose added mouse sera and control sera. Significantly increased percentages of inducible nitric oxide synthase (iNOS) and indoleamine 2,3-dioxygenase (IDO) producing MDSCs and dendritic cells (DC) were found in the spleens of diabetic mice prior to tumor induction. 1-*methyl*-DL-*tryptophan*, specific IDO inhibitor, almost completely restored phenotype of NK cells cultivated in diabetic sera. These findings indicate that diabetes promotes breast cancer growth at least in part through increased accumulation of immunosuppressive cells and IDO mediated attenuation of NK cells.

P.B2.05.08

IL-20 promoted tumor growth in hepatocellular carcinoma

Y. H. Hsu[1,2], *M. S. Chang*[3];
[1]*Institute of Clinical Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan,* [2]*Research Center of Clinical Medicine, National Cheng Kung University Hospital, Tainan, Taiwan,* [3]*Department of Biochemistry and Molecular Biology, College of Medicine, National Cheng Kung University, Tainan, Taiwan.*

Introduction: IL-20 is a proinflammatory cytokine involved in rheumatoid arthritis, atherosclerosis, and osteoporosis. However, the role of IL-20 in hepatocellular carcinoma (HCC) is unclear. We explored the function of IL-20 in HCC.Materials and Methods: Tumor tissue samples were analyzed the expression of IL-20 and cyclin D1 by using immunohistochemistry staining and quantitative real-time polymerase chain reaction analysis. To examine the role of anti-IL-20 monoclonal antibody in tumor growth, BALB/c mice was injected with ML-1 cells and treated with anti-IL-20 monoclonal antibody. Results: HCC tumor tissue expressed higher levels of IL-20 than did non-tumor tissue. High IL-20 expression in HCC was correlated with poor overall survival. IL-20 and cyclin D1 expression were also highly correlated in HCC patient specimens and 3 human HCC cell lines. IL-20 also increased cell proliferation and migration, and it regulated matrix metalloproteinase (MMP)-13, tumor necrosis factor (TNF)-α, cyclin D1, and p21WAF1 expression in ML-1 cells. Anti-IL-20 monoclonal antibody attenuated tumor growth in mice inoculated with ML-1 cells. The expression of cyclin D1, TNF-α, MMP-9, and vascular endothelial growth factor was significantly inhibited after anti-IL-20 monoclonal antibody treatment. Conclusions: IL-20 plays a role in the tumor progression of HCC. IL-20 might be a useful predictive marker for HCC progression.

P.B2.05.09

ACKR2 in hematopoietic precursors as a checkpoint of neutrophil release and anti-metastatic activity

M. Massara[1,2], *O. Bonavita*[1,2], *B. Savino*[1,2], *N. Caronni*[1,2], *V. Mollica Poeta*[1,3], *M. Sironi*[1], *E. Setten*[1,2], *C. Recordati*[4], *L. Crisafulli*[1], *F. Ficara*[1], *A. Mantovani*[1,3], *M. Locati*[1,2], *R. Bonecchi*[1,3];
[1]*Humanitas Research Hospital, Rozzano, Italy,* [2]*University of Milan, Milan, Italy,* [3]*Humanitas University, Rozzano, Italy,* [4]*Fondazione Filarete, Milano, Italy.*

96 Normal 0 14 false false false IT X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Tabella normale"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin:0cm; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:"Calibri",sans-serif; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-fareast-language:EN-US;} Atypical chemokine receptors (ACKRs) are regulators of leukocyte traffic, inflammation, and immunity. ACKR2 is a scavenger for most inflammatory CC chemokines and is a negative regulator of inflammation. We reported that ACKR2 is expressed in hematopoietic precursors and downregulated during myeloid differentiation. Genetic inactivation of ACKR2 results in increased levels of inflammatory chemokine receptors and release from the bone marrow of neutrophils with increased anti-metastatic activity. In a model of NeuT-driven primary mammary carcinogenesis ACKR2 deficiency is associated with increased primary tumor growth and protection against metastasis. ACKR2 deficiency results in neutrophil-mediated protection against metastasis in mice orthotopically transplanted with 4T1 mammary carcinoma and intravenously injected with B16F10 melanoma cell lines. Thus, ACKR2 is a key regulator (checkpoint) of mouse myeloid differentiation and function and its targeting unleashes the anti-metastatic activity of neutrophils in mice. <!--EndFragment-->

P.B2.05.10

Factor H (FH) binding and elevated expression levels of membrane complement regulators (mCRP) are key players of enhanced complement resistance of breast cancer cells upon treatment with Paclitaxel and Doxorubicin

M. H. Nasrh[1], *M. Kirschfink*[1,2];
[1]*Institute of Immunology, 69120-Heidelberg, Germany,* [2]*National Research Centr (NRC), Cairo, Egypt.*

Tumor resistance to chemotherapy is a major problem in cancer treatment. Resistance exists against every effective anticancer drug and can develop by numerous mechanisms including decreased drug uptake, increased drug efflux, activation of detoxifying systems, activation of DNA repair mechanisms, evasion of drug-induced apoptosis, etc. There are contrasting data on a possible correlation between the level of expression of multidrug resistance (MDR)-associated drug transporter P-glycoprotein (P-gp) and the susceptibility to complement-dependent cytotoxicity (CDC). Our previous studies revealed that enhanced resistance of chemo-selected MDR ovarian carcinoma cells to CDC is not conferred by P-gp, but due at least partly to overexpression of mCRP. We here investigated the sensitivity of the human breast carcinoma cell lines SKBR3 and BT474 to complement-dependent cytotoxicity in response to short-term treatment with Paclitaxel and Doxorubicin. Drug treated carcinoma cells showed increased resistance to CDC associated with an overexpression of membrane-bound complement regulatory proteins, CD46, CD55 and CD59. Drug treatment of carcinoma cells also induced the release of the soluble regulator Factor H with secondary binding to the drug-treated cell as well as the production of C1 inhibitor and factor I.
We conclude that the release of multiple soluble complement regulators into the microenvironment of breast carcinoma cells together with enhanced levels of mCRPs induced by chemotherapeutic agents even upon short-term treatment are significant to increased tumor cell resistance to complement-mediated killing.
This work is financially supported by German Academic exchange service (DAAD), through German Egyptian Research Long-Term Scholarship Program (GERLS).

P.B2.05.11

MicroRNA expression profiling in acute myeloid leukaemia patients and healthy donors according to age

*B. Sabchez-Correa[1], **A. Pera**[2], F. Hassouneh[2], C. Campos[2], N. Lopez-Sejas[2], J. M. Bergua[3], M. Arcos[3], H. Bañas[3], E. Durán[4], R. Solana[2], R. Tarazona[1];*
[1]Immunology Unit, University of Extremadura, Cáceres, Spain, [2]IMIBIC - Reina Sofia University Hospital - University of Cordoba, Cordoba, Spain, [3]Department of Haematology, Hospital San Pedro de Alcantara, Cáceres, Spain, [4]Histology and Pathology Unit, Faculty of Veterinary, University of Extremadura, Cáceres, Spain.

Introduction: MicroRNAs (miRNAs) are important regulators of biological processes such as cell proliferation/apoptosis, immune responses and tumorigenesis. miRNAs dysregulation have been identified in haematological malignancies including acute myeloid leukaemia (AML), which is a disease of older adults. Ageing has been associated with a progressive deterioration of the immune system that limits the capacity to mount an appropriate immune response to pathogens and may affect tumour immunosurveillance. Recently, age-associated changes in miRNA profiles have been described some of them related to immune system function.
Methods: we analysed miRNA expression profiles in AML patients and the effect of aging on miRNA expression.
Results: we identified six miRNAs significantly lower and seven miRNAs that were significantly upregulated in AML patients. Some of them have been implicated in cancer pathogenesis.
Conclusions: we demonstrate that AML induces changes in miRNA profile that have the potential to be diagnostic or prognostic biomarkers of disease. In addition, in healthy donors we have found three miRNAs (miR-15b, miR-181a, miR-494) that were significantly decreased with age. However, no age-associated differences were observed in AML patients suggesting that AML-induced changes in miRNA profile surpass the effect of age itself. In conclusion, circulating miRNAs in AML had a distinctive profile that distinguishes patients from healthy donors. Further characterization of circulating miRNAs in AML and the effect of age in their expression are required to use miRNAs as biomarkers of disease and ageing.
Work supported by project PI16/01615 from I+D+I National program 2013-2016 and co-funded by "ISCIII-Subdireccion General de Evaluacion" and FEDER

P.B2.05.12

Comprehensive immune landscape of a lung cancer model

***X. L. Raffo Iraolagoitia**[1], D. Reijmer[1,2], A. J. McFarlane[1], B. Kruspig[3], J. Secklehner[1,4], J. B. MacKey[1,4], S. B. Coffelt[1,3], D. J. Murphy[3,1], L. M. Carlin[1,4];*
[1]Cancer Research UK Beatson Institute, Glasgow, United Kingdom, [2]Universiteit Utrecht, Utrecht, Netherlands, [3]Institute of Cancer Sciences, University of Glasgow, Glasgow, United Kingdom, [4]Imperial College London, London, United Kingdom.

Lung homeostasis relies on resident (immune and non-immune) cells and recruited leukocytes to cope with potentially harmful agents entering the airways without causing excessive damage and chronic inflammation. Both sides of this tightly regulated balance between tolerogenic circuits and inflammatory pathways could be potentially rewired by transformed cells to create a tumour permissive microenvironment. Although immunotherapies have proven to be very successful for a number of cancers, just a small fraction of lung cancer patients benefits from them and lung cancer remains the leading cause of cancer death. Therefore, gaining further insight into how lung leukocytes support and/or antagonise tumour growth is crucial to improve therapeutic strategies. To address this, we used a conditional transgenic mouse model (lsl-KRas^{G12D}; Rosa26-lsl-MYC) that develop lung cancer upon Adeno-Cre intranasal administration. By flow cytometry, we characterized the immune cell composition and phenotype in lung, blood, spleen, bone marrow and liver, 6 weeks after induction (when all mice have developed adenocarcinoma but have not yet shown any sign of sickness) and at end point. Additionally, we studied leukocyte localisation and distribution patterns by confocal imaging of large 3D volumes within agarose inflated precision cut lung slices. Overall, we found major tumour-induced changes in the immune-landscape of the lung which may play an important role promoting tumour escape. We next aim to interrogate potential connections between these alterations to gain mechanistic information to further dissect and exploit.

P.B2.05.13

Elucidating the pathophysiological role of dendritic cells in multiple myeloma

***J. R. Richardson**[1], M. Günter[1], K. Weisel[1], H. Bühring[1], H. R. Salih[2], S. E. Autenrieth[1];*
[1]Department of Internal Medicine II, University Hospital Tuebingen, Tübingen, Germany, [2]Clinical Collaboration Unit Translational Immunology (DKTK and DKFZ), Tübingen, Germany.

Introduction: Multiple Myeloma (MM) is a B cell malignancy, characterized by clonal expansion of malignant plasma cells in the bone marrow (BM). DCs are key players of the immune response by linking the innate and adaptive immune system. The role of DCs in MM pathogenesis is poorly understood. *Methods:* We analyzed different DC subsets and progenitors *ex vivo* (n=35 MM, n=20 HD) from the BM of MM patients upon initial diagnosis and correlated the data to the clinical status. Patients subjected to hip replacement served as healthy donors (HD). Furthermore, sorted CD34$^+$ progenitor cells from MM patients and HD were analyzed for their capacity to differentiate into DC subsets *in vitro*. *Results:* The frequencies of plasmacytoid DCs (0,476% ±0,277 vs. 0,202% ±0,174) and CD141$^+$ DCs (0,121% ±0,094 vs. 0,023% ±0,028) as well as that of their immediate progenitors, "common DC progenitors" (CDPs) (0,058% ±0,0085 vs. 0,0011 ±0,0014) and pre-DCs (0,0359% ±0,0826 vs. 0,0076% ±0,0096), were significantly reduced in the BM of MM patients compared to HD, whereas the frequency of monocytes was increased. Moreover, the reduction of CD141$^+$ DCs, pre-DCs and CDPs correlated with the disease stage. Besides, CD34$^+$ progenitors from MM patients showed a reduced *in vitro* differentiation potential as well as proliferation capacity compared to that of HD. *Conclusion:* Our data indicate that DC differentiation in MM patients is intrinsically impaired most likely leading to less differentiated DCs.

P.B2.05.14

Crosstalk between pericytes and colorectal cancer cell promotes tumorigenicity through release of soluble factors

*R. Navarro[1], A. Tapia[1], M. Compte[1], A. Erce[1], M. Zonca[1], L. Alvarez-Vallina[2], **L. Sanz**[1];*
[1]Molecular Immunology Unit, Hospital Puerta de Hierro, Majadahonda, Spain, [2]Immunotherapy and Cell Engineering Laboratory, Department of Engineering, Aarhus University, Aarhus, Denmark.

Introduction: Pericytes are mural cells that surround endothelial cells in small blood vessels and constitute a source of tumor-associated stromal cells. Crosstalk between cancer cells and stroma promotes tumor progression, but the potential role of pericytes (PC) in the tumor microenvironment had not been addressed.
Materials and Methods: To analyse the potential role of PC in the tumor microenvironment, *in vitro* proliferation, migration and invasion of the human colorectal cancer cell (CRC) line HCT 116 were assayed in co-culture with primary PC. The effect of coimplantation in tumor growth was studied *in vivo* establishing a xenograft model. Transcriptomic profiling of PC after mono- or co-culture with HCT 116 was performed. Antibody arrays were used to analyse the secretome of PC in mono- or co-culture.
Results: We demonstrated that co-culture with PC enhanced HCT 116 tumorigenicity increasing tumor cell proliferation, migration and invasion. Moreover, co-implantation of HCT 116 tumor cells with PC accelerated tumor growth *in vivo*. PC cultured with HCT 116 cells expressed a distinct gene signature which correlated with clinical outcome in CRC patients. Conditioned media of co-cultured PC contained different mediators that could explain the functional effects observed in HCT 116, including IL6, IL11 and LIF.
Conclusion: We have found the crosstalk between PC and CRC cells to be bidirectional, i.e. tumor cells modulate PC gene expression that, in turn, promote pro-tumorigenic behaviour by inducing the secretion of mediators that facilitate tumor progression. This study suggests that PC deserve further investigation as potential therapeutic targets in cancer.

P.B2.05.15

Effect of Nasopharyngeal Carcinoma-derived exosomes on the emergence of tolerogenic dendritic cells

***R. Sarah**[1], C. Havet[1], Z. Fitzpatrick[2], B. Hennart[3], D. Allorge[3], O. Moralès[1], N. Delhem[1];*
[1]CNRS UMR8161, Lille, France, [2]Harvard Medical School, Boston, United States, [3]Centre de Biologie-Pathologie, CHRU de Lille, Lille, France.

Background: A characteristic of the nasopharyngeal carcinoma (NPC) micro-environment is the presence of immunosuppressive exosomes released by tumor cells. Our team has recently shown that NPC-derived exosomes favor the recruitment and suppressive activity of human regulatory T cells (Treg), thus contributing to NPC immune escape (Mrizak et al., JNCI, 2015).
Question: In this study, our objective is now to evaluate whether these NPC-derived exosomes could promote the emergence of tolerogenic dendritic cells (tolDC) able to induce regulatory T cells contributing to the tolerance of tumor cells.
Methods: We performed a complete phenotypical and functional study comparing the effect of NPC and healthy donor-derived exosomes on DC maturation. This study includes (**i**) a transcriptomic study by RTqPCR, (**iii**) flow cytometric analysis of the expression of DC phenotype and Treg markers, (**iv**) a preliminary DC functional study by western blotting of the enzyme IDO1 and HPLC dosage of tryptophan metabolites, (**v**) a secretome analysis by ELISA (**vi**) and finally a functional assay where the DC-exposed to NPC-exosomes are co-cultivated with PBMCs or naïve T cells in order to determine the type of T cells generated.
Conclusions: Taken together our results strongly suggest that the presence of NPC-derived exosomes favors the emergence of semi-mature tolDCs. Despite the importance of tolDCs in immune escape, no other study has yet shown the impact of tumor exosomes on the maturation of human DCs. Thus, these promising results should open new prospects for antitumor immunotherapies based on the inhibition of factors involved in the emergence of Tregs.

P.B2.05.16

Overexpression of the chemokine receptor CXCR3 on CD5^{high} and CD5^{low} leukemic cells correlates with favorable prognosis in chronic lymphocytic leukemia

V. Smotkova Kraiczova[1], *G. Manukyan*[2,1], *R. Fillerova*[1], *Z. Mikulkova*[1], *G. Gabcova*[1], *R. Urbanova*[3], *P. Turcsanyi*[3], *A. Petrackova*[1], *T. Papajik*[3], *E. Kriegova*[1];
[1]*Department of Immunology, Faculty of Medicine and Dentistry,Palacky University Olomouc, Olomouc, Czech Republic,* [2]*Laboratory of Molecular and Cellular Immunology, Institute of Molecular Biology NAS RA, Yerevan, Armenia,* [3]*Department of Hemato-Oncology, Faculty of Medicine and Dentistry, Palacky University and University Hospital, Olomouc, Czech Republic.*

Despite the shared pattern of surface antigens, population of neoplastic cells in chronic lymphocytic leukemia (CLL) demonstrate a heterogeneity in their molecular and functional characteristics. Given the dynamic variations of CD5 expression and its link to the proliferating pool of neoplastic cells, we aimed to further characterize chemokine and adhesion molecule profile on CD5^{high} and CD5^{low} neoplastic clones in peripheral blood of CLL patients as well as their relevance to the disease progression and patient characteristics. CD5^{high} cell subpopulation expressed higher levels of CXCR3 (P<0.001), CXCR5 (P=0.007), CCR10 (P=0.001), CD62L (P=0.029) compared with those expressed on CD5^{low} cells, whereas CD5^{low} cells expressed higher levels of CXCR4 receptor (P<0.001). Accordingly, CXCR3/CXCR4 ratio was higher on CD5^{high} comparing to CD5^{low} B-cells (P<0.001). Mutated *IGHV* status was strongly associated with higher percentage of CXCR3 cells on both CD5^{high} (P=0.001) and CD5^{low} (P<0.001) cells, as well as higher MFI of CXCR3 on both CD5^{high} (P=0.006) and CD5^{low} (P=0.005) cells. Combination of CXCR4 (MFI) - CXCR3/CXCR4 ratio or CXCR3 (MFI) - CXCR4 (MFI) was the most significant able to discriminate CD5^{high} and CD5^{low} subpopulations. Our results suggest CXCR3 as a marker which greater expression portend a favourable prognosis. Further investigation of CLL cell heterogeneity will advance our understanding of neoplastic cell biology and its links to the prognosis.

Grant support: MZ ČR VES16-32339A, IGA UP_2018_016, MH CZ-DRO (FNOL, 00098892)

Smotkova Kraiczova V*, Manukyan G*

*contributed equally

P.B2.05.17

Checkpoint inhibitors, cancer and myositis: A report of four illustrative cases

G. Vila-Pijoan[1], *M. T. Sanz-Martínez*[1], *L. Viñas-Giménez*[1], *J. Ros*[2], *J. Lostes-Bardaji*[2], *A. Navarro*[2], *F. Martínez-Valle*[3], *V. García-Patos*[4], *C. Carpio*[5], *J. C. Milisenda*[6], *J. M. Grau*[6], *R. Pujol-Borrell*[1,7,8], *A. Selva-O'Callaghan*[3,9];
[1]*Immunology Division, Vall d'Hebron University Hospital (HUVH), Barcelona, Spain,* [2]*Vall d'Hebron Institute of Oncology (VHIO), Universitat Autònoma de Barcelona, Barcelona, Spain,* [3]*Systemic Autoimmune Diseases Unit, Vall d'Hebron University Hospital (HUVH), Universitat Autònoma de Barcelona, Barcelona, Spain,* [4]*Dermatology Dept, University Hospital Vall d'Hebron (HUVH), Barcelona, Spain,* [5]*Hematology Dept, University Hospital Vall d'Hebron (HUVH), Barcelona, Spain,* [6]*Research Muscle Unit, Hospital Clínic de Barcelona, Universitat de Barcelona, Barcelona, Spain,* [7]*Diagnostic Immunology Group, Vall d'Hebron Research Institute (VHIR), Barcelona, Spain,* [8]*Cell Biology, Physiology and Immunology Dept, Universitat Autònoma de Barcelona, Barcelona, Spain,* [9]*Medicine Dept, Universitat Autònoma de Barcelona, Barcelona, Spain.*

Introduction: Immunotherapy aimed at blocking PD1/PDL1 pathway restores anti-tumor immunity but may result in a number of Immune-Related Adverse Events (iRAE).
Patients and Methods: Three patients, receiving PD1/PDL1 blockers (Cases 1 to 3) were referred to internal medicine because of symptoms suggestive of myopathy. Case 4 was already diagnosed as dermatomyositis. Clinical, immunological and histopathological evaluation confirmed immune-mediated myositis.
Results: Case 1 was a breast carcinoma who 24 hours after the first cycle of anti-PD1 (Pembrolizumab) presented with asthenia and a macular rash in both ankles followed 3 months later by neutropenia, hypothyroidism and amyopathic dermatomyositis. Case 2 was a malignant thymoma treated with anti-PDL1 (Atezolizumab) who developed immune-mediated necrotizing myopathy negative for myositis auto-antibodies (16 antigens). Case 3 was a non-Hodgkin lymphoma receiving anti-PD1 (Nivolumab) who developed sporadic inclusion body myositis with anti-CN1A. In cases 1 and 2 the suspension of immune-checkpoint therapy after 11 and 4 cycles respectively plus steroids induced remission of myositis. In Case 3 treatment interruption did not result in improvement. Case 4 was already diagnosed of paraneoplastic dermatomyositis associated to a primary small-cell lung carcinoma positive for anti-NXP2 prior to anti-PD1 (Nivolumab). Interestingly anti-PD1 was well tolerated with a partial response of the tumour without exacerbation of the dermatomyositis.
Conclusion: Immune mediated myositis has been reported in very few patients on checkpoint inhibitor therapy. Skin lesions and muscle weakness should alert and prompt for effective management. On the other hand, the presence of paraneoplastic dermatomyositis is not a contraindication of checkpoint inhibitors treatment.

P.B2.05.18

Identification of a stromal stem cell in pancreatic cancer that drives tumor growth and metastasis

Z. Wu[1,2,3], *X. Zhang*[1,2,3], *Y. Zhao*[1,2,3], *I. Mikaelian*[1,2,3], *P. Bertolino*[1,2,3], *A. Hennino*[1,2,3];
[1]*Cancer Research Center of Lyon, UMR INSERM 1052, CNRS 5286, F-69373, Lyon, France,* [2]*Université Lyon 1, F-69000, Lyon, France,* [3]*Centre Léon Bérard, F-69008, Lyon, France.*

Introduction: Cancer stem cells (CSCs) have been characterized as the properties of stem-like features, tumorigenicity and treatment-resistant. Investigations of CICs have been manipulated in pancreatic ductal adenocarcinoma (PDAC), in which tumor microenvironment plays an important role in tumor regulation. However, the identification of pancreatic CICs within native pancreatic tumor microenvironment still remains unclear.Materials and Methods:KC (p48-Cre;K-Ras$^{+/LSLG12D}$) and KIC(Ptf1/p48-Cre; K-RAS$^{+/LSLG12D}$; Ink4a/Arf$^{flox/lox}$) mice model, cell culture, immunohistochemistry and immunofluorescence, FACS, RT-qPCR, subcutaneous injection into Rag KO mice.Results: A subpopulation of cells in pancreas of the KC mice defined as CD45-EpCAM-PDGFRα-CD24+CD44+ have a stromal localization and stromal signature (Fsp1↑, Vim↑, Actα2↑), but no epithelial gene expression (CK19↓, CK7↓, Sox9↓). Moreover, this subpopulation shares some features of embryonic stem cells (Oct3/4) and adult stem cells (Nestin). Furthermore, Alk4 (Activin A cognate receptor) and Alk5 (TGF-βR1 receptor) expression is significantly higher in CD24+CD44+subpopulation compared to CD24-CD44-counterpart suggested the involvement of TGFβ/ActivinA signaling pathway in regulation of CD24+CD44+subpopulation. The in vivo co-injection of this stromal population along with transformed epithelial Kras primary cell line leads to increase tumor progression and metastasis. Surprisingly, we also discover that the cell and spatial density is highly related with their degree of differentiation and outcome both in vitroand in vivo.Conclusion: We have identified a population of stromal stem cells generated from KC mice that might be at least in part at the origin of the desmoplastic reaction in PDAC. Further in vivo and in vitro investigation will provide new hints into the mechanism of generation of the stromal reaction.

P.B2.05.19

Role of carbohydrate metabolism abnormality in non-small cell lung cancer-related immunoparalysis

Z. I. Komlósi[1,2,3], *G. Szücs*[2], *É. Imre*[3], *M. Szentkereszty*[4], *G. Barna*[4], *G. Losonczy*[2];
[1]*Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary,* [2]*Department of Pulmonology, Semmelweis University, Budapest, Hungary,* [3]*Department of Laboratory Medicine, Semmelweis University, Budapest, Hungary,* [4]*1st Dept. of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary.*

Human Leukocyte Antigen-DR (HLA-DR), a major histocompatibility complex class II cell surface receptor, is involved in antigen presentation to T helper cells, and its reduced expression on monocytes is associated with poor prognosis in non-small cell lung cancer (NSCLC; Lancet Oncol 2013. 14:e218). Both elevated fasting serum glucose level (> 7 mmol/L) and diagnosed diabetes mellitus are independent negative prognostic markers in NSCLC (Lung Cancer 2012. 76:242), however the mechanisms by which the abnormality of carbohydrate metabolism aggravate lung cancer have not been fully clarified. Hyperglycemia and insulin resistance are known to induce a reduction in HLA-DR expression (immunoparalysis) in sepsis. We aimed to investigate the role of carbohydrate metabolism abnormality in NSCLC-related immunoparalysis. 33 NSCLC patients (stage IIIb-IV) were included in the study. Monocyte HLA-DR expression was measured by using flow cytometry. Serum glucose and insulin concentration was measured and Homeostasis Model Assessment - Insulin Resistance 2 (HOMA-IR2) score was calculated. 18 patients had hyperglycemia, and 16 out of them were insulin resistant (HOMA-IR2 > 2). A significant indirect correlation was revealed both between insulin concentration and HLA-DR expression, and between HOMA-IR2 and HLA-DR expression. Our results suggest that insulin resistance may contribute to the unfavorable alteration of antigen presentation capacity, and consequently, an insufficient anti-tumor immune response in NSCLC. This underline the previously unappreciated importance of the management of carbohydrate homeostasis in lung cancer patients. Funded by NKFIH K 108009.

P.B2.05.20

Monitoring antibody responses against tumour associated protein mutations might clinically be of prognostic relevance

H. Thiesen[1], *F. Steinbeck*[1], *E. Schade*[2], *M. Maruschke*[3], *O. Hakenberg*[4];
[1]*Institute of Immunology, Rostock, Germany,* [2]*Gesellschaft für Individualisierte Medizin, Rostock, Germany,* [3]*Helios Hanseklinikum Stralsund, Stralsund, Germany,* [4]*Department of Urology University Medicine Rostock, Rostock, Germany.*

Introduction: Whole somatic genome sequences of three patients suffering from clear cell renal cell carcinoma (ccRCC) have been computationally analysed to categorize protein structures mutated in these tumors. The working hypothesis addressed is focussed on the prognostic relevance (harmful or beneficial) of elicited tumor-associated antibody reactivities. Method: Peptides representing putative neoepitopes such as point as well as frame shift mutations were synthesized. Initially, peptide microarrays were used to select mutated peptides that show epitope-antibody-reactivities (EAR) in sera of ccRCC patients. The ten most informative peptides were finally subjected to MSD multi-arrays by addressing 10 different peptides per well. Results: Of 94 ccRCC sera tested, 20 sera showed at least one positive signal, of which 8 sera reacted with more than one peptide selected from our initial screen. Interestingly, the patient that show EAR with all 10 preselected peptides turned out to suffer from metastatic ccRCC. He died shortly after his tumour had been removed. Conclusion: Our multi-array analysis of tumour associated antibodies directed against mutated peptide sequences present in patients suffering from clear cell renal carcinoma indicate that the presence of antibodies directed against tumor related protein structures might have a negative prognostic effect.

In turn, peptide vaccination strategies should be carefully handled as long as they lead to enhanced humoral antibody responses. At the moment, we cannot exclude that these antibody specificities elicited might counterbalance current cellular immune therapies that stop ongoing checkpoint inhibitions. Multicentric studies await to be conducted to validate the clinical and prognostic value of EAR against mutated protein structures.

P.B2.05.21
Therapeutic induction of tertiary lymphoid structures in tumors

G. Vella[1], *O. Burton*[2], *Y. Hua*[1], *E. Allen*[1], *M. Duhamel*[1], *S. Schlenner*[2], *G. Bergers*[1,3];
[1]*Laboratory of Tumor Microenvironment and Therapeautic Resistance, VIB-KU Leuven Center for Cancer, Leuven, Belgium,* [2]*Laboratory of Genetics of Autoimmunity, VIB-KU Leuven Center for Brain & Disease Research, Leuven, Belgium,* [3]*3. Department of Neurological Surgery, Brain Tumor Research, Helen Diller Family, Comprehensive Cancer Center, University of California, San Francisco, United States.*

Tertiary lymphoid structures (TLSs) are induced postnatally in non-lymphoid tissues such as those affected by chronic infections or autoimmune diseases, but also spontaneously form in cancer tissues. They are composed of surrounding stromal and lymphoid cell constituents without the necessity of a capsule and contain high endothelial venules (HEV) that are adapted for lymphocyte trafficking and normally found in lymph nodes. While TLS formation in inflamed tissues is associated with a worse outcome, spontaneous TLS formation in several human cancer types correlated with improved patient survival. We have recently shown that intratumoral HEV formation can be therapeutically induced by antiangiogenic immunotherapy in mouse models of pancreatic and breast cancer, and is dependent on lymphotoxin-beta receptor activation. Intratumoral HEV formation enhanced T-and B-cell influx forming TLS-like structures, which subsequently inhibited tumor growth and enhanced survival. Using powerful analytical tools we are currently evaluating the immune composition and immune functionality in HEV+ tumors (by FACSymphony™) and specifically around HEV (by spatial transcriptomics and immunofluorescence staining) in a murine breast cancer model. Our data provide evidence that therapeutically induced TLS/HEVs in tumors generate an immunostimulatory microenvironment by specifically increasing the levels of intratumoral activated CD8+, CD4 Th1+ and NK cells.

P.B2.06 Environmental regulation anti-tumor responses - Part 6

P.B2.06.01
The regulatory effect of Type I IFNs on neutrophil angiogenic capability in tumor microenvironment

S. Bordbari[1], *E. Pylaeva*[1], *I. Spyra*[1], *I. Helfrich*[2], *S. Lang*[1], *J. Jablonska*[1];
[1]*Research division of Otorhinolaryngology department of University Hospital Essen, Essen, Germany,* [2]*Skin Cancer Unit of the Dermatology Department of University Hospital Essen, Essen, Germany.*

Introduction: Tumor-associated neutrophils (TANs) influence tumor growth and angiogenesis, depending on IFNs availability in milieu. Since angiogenesis plays a crucial role in tumor progression, the aim of this study is to determine the mechanism responsible for neutrophil-dependent tumor vascularization, and how IFNs impact this process. **Methods:** First, we analyzed TAN infiltration, vasculature development and maturation stages of endothelial cells in the tumors. We assessed also hypoxic areas and the vessel leakiness in tumors. Pro-angiogenic regulatory gene expression was analyzed in endothelial cells after stimulation with TANs. Additionally, we assessed the capacity of TANs to stimulate proliferation, migration, tube formation assay and sprout-forming capacity of endothelial cells. To delineate the role IFNs play in the regulation of tumor angiogenesis, we compared TANs from IFN-deficient mice with WT. **Results:** We could observe elevated tumor growth and higher TAN numbers in IFN-deficient mice which were associated with more mature, functional phenotype tumor vasculature and show significant upregulation of pro-angiogenic factors, such as Neuropilin1 (NRP1) and Endoglin (ENG). Interestingly, these tumors show also increased hypoxia. Co-culture of endothelial cells with such proangiogenic IFN-deficient TANs led to profound increase of their proliferation, migration, tube formation and sprouting capacity. In agreement, aortic ring assay showed more microvessel outgrowths after addition of IFN-deficient TANs. **Conclusion:** Taken together, our results suggest that IFN-deficient TANs of pro-angiogenic phenotype can efficiently stimulate tumor vascularization and tumor growth. The lack of type I IFNs stimulates their proangiogenic capacity due to upregulation of Pro-angiogenic regulatory genes.

P.B2.06.02
Cytotoxic effects of tamoxifen on MCF-7 cells may be improved by propolis without affecting the viability of human monocytes

E. O. Cardoso[1], *K. B. Santiago*[1,2], *B. J. Conti*[1], *F. L. Conte*[1], *L. P. Oliveira*[1], *K. I. Tasca*[1], *J. M. Sforcin*[1];
[1]*Department of Microbiology and Immunology, Biosciences Institute, Sao Paulo State University, Botucatu, Brazil,* [2]*Integrated Regional Faculties of Avaré, Avaré, Brazil.*

Cancer is a disease that affects millions of people in different continents, resulting in millions of deaths annually. Tamoxifen has been used to treat breast cancer and considering the side effects caused by anticancer agents, such as toxicity, induction of resistance in tumor cells and immunosuppression, the administration of natural products simultaneously with anticancer drugs has been investigated. Propolis is a bee product displaying a cytotoxic activity against tumor cells and modulatory effects on immune cells. This study investigated the cytotoxic effects of propolis in combination with tamoxifen (P + TAM) on MCF-7 cells and on human monocytes. MCF-7 cells were used as a tumoral model and were treated with tamoxifen (0.25, 0.5, 1 and 2.5 μM), propolis (25, 50, 75 and 100 μg/mL), and their combinations for 24, 48 and 72 h. Human monocytes were obtained from 5 healthy donors and treated with the same concentrations for 18 h. After these periods of time, cell viability was assessed by the colorimetric MTT assay. Significant differences between treatments were determined by analysis of variance (ANOVA), followed by Dunnett's test ($P < 0.05$). Among all treatments and concentrations, propolis (50 and 75 μg/mL) increased the cytotoxic action of tamoxifen (0.25 and 0.5 μM) against MCF-7 cells after 48 h without affecting monocyte viability. This finding indicated that these combinations may be efficient against tumor cells *in vitro* but not normal ones, preserving monocytes functions. Finnancial support: FAPESP 2016/09986-4.

P.B2.06.03
IFN-gamma signaling involved in PD-L1 expression in tumor associated macrophages of mouse orthotopic liver cancers treated with sorafenib

C. Chang[1], *S. Yang*[1], *C. Hsu*[1,2];
[1]*Graduate Institute of Oncology, College of Medicine, National Taiwan University, Taipei, Taiwan,* [2]*Department of Oncology, National Taiwan University Hospital, Taipei, Taiwan.*

Introduction: We previously demonstrated that PD-L1 expression increased in tumor-associated macrophages (TAMs) of hepatocellular carcinoma (HCC) tumors progressing from sorafenib treatment. The current study explored the potential mechanisms underlying the increased PD-L1 expression in TAMs of HCC.
Materials and Methods: BNL cells, a mouse liver cancer cell line, were implanted on liver of BALB/c mice, and were fed with sorafenib (5 mg/kg/day) for 1 week. Specific antibodies were used to detect the expression of PD-L1, CD11b, F4/80, CD86, or MHC II in TAMs. RAW 264.7 and PMA-treated THP-1 cells were primed with IL-4, IL-10, IL-13 and TGF-β, followed by treating IFN-γ plus TNF-α or LPS. The expression of genes of interest was quantitated by Syber Green Real-Time PCR method.
Results: Sorafenib treatment suppressed the growth of BNL mouse liver tumors. $CD11b^+F4/80^+$ TAMs (50-73%) from sorafenib- treated liver tumors exhibited increased expression of PD-L1 (MFI 316.8), CD86 (MFI 136.3), and MHC II (MFI 341.7). Transcripts analysis not only confirmed the increased expression of PD-L1, CD86 and MHC II, but also revealed the upregulation of IFN-γ signaling including iNOS, IL-12, Stat1, IRF1, and JAK1/2 in TAMs from sorafenib-treated liver tumors. In cultured RAW 264.7 or THP-1 cells with M2 polarization, we found that IFN-γ/ TNFα or IFN-γ/ LPS upregulated the expression of PD-L1, CD86, and MHC II.
Conclusions: Our data suggest that activation of IFN-γ signaling contributes to PD-L1 upregulation in TAMs of mouse liver cancer treated with sorafenib.

P.B2.06.04
Food-derived β-glucans: polarization towards M1-like macrophages

P. de Graaff[1,2], *A. van Laar*[2], *M. M. Tomassen*[2], *C. Berrevoets*[1], *R. Debets*[1], *C. Govers*[2];
[1]*Erasmus MC-Cancer Institute, Rotterdam, Netherlands,* [2]*Food & Biobased Research, Wageningen, Netherlands.*

Introduction: Immune therapies have shown clear clinical effects in the treatment of solid tumors. Despite significant initial responses, these therapies are currently challenged by incomplete and non-durable responses in the majority of patients, which are in part related to T cell evasive mechanisms. In the current study, we assess non-digestible polysaccharides (ß-glucans) for their 'adjuvant effect' towards innate immune cells to support anti-tumor effects of T cells.
Materials and methods: Nine β-glucans (Maitake D-fraction, Oat, Zymosan, Lentinan, Curdlan, Schizophyllan, Whole Glucan Particles and two types of yeast-derived ß-glucans) were tested for their effects on the plasticity of human monocyte-derived macrophages (M0) or macrophages that were first polarized to M2 macrophages. The phenotype and function of resulting macrophages was assessed by qPCR, whole genome expression analysis, flow cytometry and ELISAs, as well as tumor cell:T cell:macrophage co-culture experiments.
Results: Zymosan, Yeast Immitec and Curdlan up-regulated gene expression of CCR7, ICAM-1 and CD80, and significantly increased the secretion of both TNFα and IL-6 in M0 macrophages. Notably, when starting from immune suppressive M2-like macrophages, often prevalent in solid tumors, these three β-glucans again pushed macrophages towards an M1-like phenotype. Moreover, these β-glucans induce expression of T cell selective chemoattractants, and were able to reverse macrophage-mediated inhibition of anti-tumor T cell responses.
Conclusion: These *in vitro* analyses demonstrate that selected β-glucans have the unique ability to preferentially skew macrophages towards an M1-like, T cell supportive, phenotype.

P.B2.06.05

Characterisation of novel CD73 antibodies as a therapeutic method of adenosine regulation

G. Gernon[1], *S. Grooby*[1], *L. Tonkin*[1], *A. Bitterwolf*[2], *L. Stewart*[2], *P. Shah*[1], *Z. Johnson*[1], *K. Ewings*[1];
[1]*Cancer Research UK Therapeutic Discovery Laboratories, Cambridge, United Kingdom,* [2]*Cancer Research UK Therapeutic Discovery Laboratories, London, United Kingdom.*

CD73 is a membrane-bound nucleotidase receptor which is frequently overexpressed in the tumour microenvironment and can be found on both tumour and infiltrating immune cell. Its function is to catalyse the conversion of adenosine monophosphate (AMP) to adenosine and phosphate and it has been proposed as a therapeutic target in cancer due to the role of adenosine in tumour immune suppression.
A series of novel CD73 antibodies have been characterised *in vitro* using multiple approaches. Inhibition of CD73 activity was evaluated using an Amplex Red-based coupled adenosine assay against both human and mouse CD73, and kinetics of antibody binding were determined using BioLayer Interferometry. Cellular assays were then utilised to further evaluate the antibodies *in vitro*. The ability of the CD73 antibodies to internalise was evaluated using two different methods, a Fab-ZAP killing assay and the IncuCyte™ FabFluor internalisation assay. The antibodies have also undergone functional studies that investigate the ability of the CD73 antibodies to disrupt the production of adenosine in tumour cells.
We demonstrate that amongst our panel of antibodies inhibit CD73 function by two different mechanisms, direct inhibition of enzyme activity and modulation of cell surface expression; both of which have therapeutic potential to disrupt CD73-mediated adenosine production and therefore reduce anti-tumor immune responses. Several antibodies from this panel will be advanced into late-stage preclinical development to identify a clinical development candidate.

P.B2.06.06

Expression of the P2X7(k) splice variant by 4T1 breast cancer cells inhibits tumor growth *in vivo*

S. Javed, *F. Koch-Nolte, F. Haag;*
University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Immunogenic cell death caused by chemotherapy can favor T cell activation by the release of damage-associated molecular patterns, including ATP. Various tumor and immune cells express the ATP-gated P2X7 ion channel. Tonic activation of P2X7 on tumor cells benefits tumor growth by stimulating cell metabolism, but strong stimulation of P2X7 can kill tumor cells. Expression of P2X7 by host immune cells is detrimental to the tumor because it enhances the anti-tumor immune response. Little is known about the role of P2X7 in the context of chemotherapy. We therefore studied the effects of P2X7 stimulation on Doxorubicin (DOX) toxicity in lymphoma and breast cancer cell lines.
Low doses of extracellular ATP (eATP) synergistically enhanced the sensitivity to DOX. This effect was linked to a specific splice variant (P2X7k) of P2X7, and was further enhanced by an agonistic nanobody to P2X7. Mechanistically, gating of P2X7 augmented the initial uptake of DOX into cells. However, enhanced cell death was also observed when DOX was washed away before exposure to eATP, suggesting that the synergism resulted from an interaction of downstream signaling pathways. In the 4T1 breast cancer model expression of P2X7 inhibited tumor growth *in vivo* by increasing apoptosis and enhancing immune cell infiltration. Our results suggest that expression of P2X7 by tumor cells may contribute to their sensitivity to chemotherapeutic drugs.

P.B2.06.07

Hypoxia regulates the fate of γδ T cells in tumour microenvironment

S. Kashipathi Sureshbabu[1,2], *A. D'Cruz*[3,2], *D. Chaukar*[3,2], *S. V. Chiplunkar*[1,2];
[1]*Advanced Centre for Treatment, Research and Education in Cancer, Navi- Mumbai, India,* [2]*Homi Bhabha National Institute, Mumbai, India,* [3]*Tata Memorial Hospital, Mumbai, India.*

Oral cancer is the most common cancer in India with relatively poor prognosis. Hypoxia is one of the factors important in predicting survival,contributing to tumour progression, therapy resistance and poor clinical outcome. Understanding the complexity of tumour microenvironment(TME) is important for the development of immunotherapy. γδ Tcells infiltrate tumours and exhibit potent antitumor activity, hence are becoming the attractive candidates for cancer immunotherapy. In the current prospective study, we aimed at investigating the effect of hypoxia on the effector functions of γδ Tcells. γδ Tcells showed increased oral tumour infiltration, exhibited marked differences in the expression of activation markers CD69 and CD25, effector molecules-perforin and IFNγ. Enhanced expression of hypoxia inducible factor1-α was observed in γδ Tcells in oral tumours. Freshly isolated γδ Tcells from healthy individuals were stimulated with αCD3mAb and 1-Hydroxy-2-methyl-2-buten-4-yl4-diphosphate(HDMAPP) and cultured in the presence or absence of hypoxia. Under hypoxia, antigen specific proliferation and activation status of γδ Tcells was unaltered. A marked decrease was observed in the anti-tumour cytotoxic ability of γδ Tcells owing to the decreased expression of IFNγ, CD107a and transcription factors Eomes and Tbet which are responsible for regulating the cytotoxic effector functions. Under hypoxia, γδ Tcells express increased RORγt and secreted cytokines favouring γδT17 differentiation. Gene expression studies of hypoxia exposed γδ Tcells confirmed γδT17 differentiation to a pro-tumor phenotype promoting angiogenesis. In conclusion, we demonstrate that hypoxic TME gives survival advantage to γδT17,thus promoting immune evasion. Funding - Terry Fox International Research Grant, The Terry Fox Foundation

P.B2.06.08

Targeting Myeloid-Derived Suppressor Cells (MDSC) in Myelodysplastic Syndromes (MDS).

J. Liu, *X. Chen, E. Eksioglu, S. Wei;*
Moffitt Cancer Center, Tampa, United States.

The acquisition of genetic abnormalities that lead to ineffective hematopoiesis is a characteristic of MDS. This event is associated with inflammatory bone marrow (BM) microenvironment; however, the underlying mechanisms are unclear. We have identified that myeloid derived suppressor cells (MDSC) are key driver of MDS progression. We found that MDSCs accumulated in excess in the BM of patients with MDS compared to controls and non-MDS cancer patients. FISH was performed to determine whether MDSCs represent a distinct cell population from the abnormal MDS clone. MDSCs chromosomal abnormality were separated by FACS sorting based on MDSC phenotype and the presence of chromosomal abnormalities was determined in this population and compared to non-MDSCs. Chromosomal abnormalities resided within the non-MDSC hematopoietic compartment indicating that MDSCs in MDS patients may represent a unique cell population from the HPCs with clonal potential. Furthermore, the key cytokines involved in MDSC suppressive function were higher in MDS patients compared to controls.
It was observed that the accumulation of MDSC in the BM from MDS patients has an impact on hematopoietic differentiation. Based on these findings, a novel form of adaptive immunotherapy based on the induction of MDSC maturation can be envisioned. DAP12, an adaptor protein, mediates signaling of myeloid cell maturation, were genetically modified to be a constitutively activated form. Infection of BM-MNC from MDS patients with constitutively active DAP12 increased expression of maturation surface markers and increased BFU-E colony formation after 14 days. These results suggest that activation of DAP12 has potential therapeutic implications in MDS.

P.B2.06.09

Epigenetic alteration of the PD-1/PD-L1 axis, a novel target for pharmacotherapy?

D. McKernan, *C. Hennessy, F. Quirke, G. O'Malley, A. Ryan;*
National University Ireland Galway, Galway, Ireland.

Introduction: Programmed death ligand 1 (PD-L1) is the primary ligand of the receptor programmed death-1 (PD-1), a coinhibitory cell surface immunoglobulin important in development of self tolerance. PD-L1 expression can be exploited by various cancers as a means of immune evasion with high PD-L1 expression associated with a poorer prognosis. Checkpoint inhibitors which target the PD-1/PD-L1 axis have shown some promise in the clinic but there is a lack of knowledge on the precise mechanisms by which PDL1 expression is regulated. Epigenetic modifications are known to alter gene expression, we therefore hypothesized that the DNA methylation and/or histone acetylation may alter PD-L1 expression. **Methods**: We used the colorectal cancer HCT116 cell line with genetically deleted DNA methyltransferase (DNMT) enzymes to determine the effect of DNA methylation on basal mRNA by QPCR and protein expression by flow cytometry. Using a number of microsatellite instable human colonic carcinoma cell lines to induce PD-L1 expression with the TLR3 ligand Poly I:C (10 μg/ml, 24 hrs) we determined the effect of both DNMT (decitabine 500 nM, 72 hrs) and histone deacetylase (SAHA, 10 μM) inhibitors on induction. **Results**: Genetic knockout of DNMT enzymes significantly reduced the basal expression of PDL1. Decitabine but not SAHA prevented Poly I:C-induced upregulation of PD-L1 mRNA and protein. Using a NF-κB superrepressed (SR) cell line, we determined that PD-L1 induction was NF-κB dependent and decitabine treatment prevented this induction. **Conclusion**: These data suggest that epigenetic modifying drugs may act as immunodulators and potentially form part of future PD-1/PD-L1 therapies.

P.B2.06.10

Development of a syngeneic mouse model of leukemia minimal residual disease: a new tool to study the involvement of the immune response in cancer cell persistence and test new immunotherapeutic strategies

A. MOPIN[1], *B. QUESNEL*[1], *C. BRINSTER*[2];
[1]*INSERM UMR-S-1172, CHU LILLE, UNIVERSITE LILLE, LILLE, France,* [2]*INSERM UMR-S-1172, UNIVERSITE LILLE, LILLE, France.*

Acute myeloid leukemia (AML) is a clonal disorder characterized by blocked differentiation and extensive proliferation of hematopoietic progenitors/precursors. Relapse is often observed after chemotherapy due to the presence of residual leukemic cells, also called minimal residual disease (MRD). Several studies have demonstrated that sub-clonal heterogeneity at diagnosis could be responsible for MRD, with major or minor sub-clones resisting chemotherapy or emerging after treatment. However, these studies do not provide information about the contribution of the immune system (elimination, control or escape) in these leukemic cell persistence or sub-clones hierarchy.

To generate this mouse model, we used well-characterized sub-clones of the murine C1498 cell line and selected them according to their *in vitro* aracytidine (Ara-c) sensitivity. These cells were stably transfected with a *ZsGreen* reporter gene to facilitate *in vivo* tracking. We tested different conditions of Ara-c administrations (concentrations, number and timing of injections). One protocol led to approximately 50% survival over an average of 90 days after non-treated control mice succumbed to AML (mean survival, 29-31 days). The assessment of residual leukemic cells in the blood of surviving mice at different time points (between 49 to 75 days after control mice died from AML) revealed the presence of 0.1 to 4.3% circulating ZsGreen$^+$ cells by flow cytometry without any signs of AML relapse. Thus, we have developed a mouse model of leukemia MRD that should offer valuable insights into the biology of residual leukemic cells and the immune mechanisms leading to their persistence, thereby enabling the development of new therapies.

P.B2.06.11

The gastrointestinal tract tumour microenvironment differentially influences maturation of and cytokine secretion from dendritic cells

M. Morrissey[1], *M. Dunne*[1], *R. Byrne*[1], *N. Lynam-Lennon*[1], *S. Kennedy*[1], *C. Nulty*[1], *N. McCabe*[1], *C. Butler*[2], *D. O'Toole*[3], *E. Ryan*[2], *J. V. Reynolds*[3], *J. O'Sullivan*[1];
[1]*Trinity College Dublin, Dublin, Ireland,* [2]*University College Dublin, Dublin, Ireland,* [3]*St James's Hospital, Dublin, Ireland.*

Oesophageal adenocarcinoma (OAC) and rectal adenocarcinoma are treated with neoadjuvant chemoradiotherapy in order to reduce tumour size prior to surgery however only 10-30% of patients have a complete pathological response. Inflammatory and angiogenic mediators in the tumour microenvironment (TME) have many functions, such as enabling evasion of anti-tumour immune responses by disabling infiltrating dendritic cells (DCs) and have been linked with radioresistance. Tumour Conditioned Media (TCM) from colonic cancer has been shown to strongly inhibit DC maturation. Our aim was to understand if this DC inhibition extends to other cancers of the gastrointestinal tract, to investigate if radiotherapy influences this and to profile constituents of TCM that may influence DC maturation.
Here we found that monocyte-derived DCs remained responsive to LPS following pre-treatment with OAC cell line TCM, whereas inhibition was induced by CRC cell line TCM. *ex vivo* TCM from different gastrointestinal adenocarinoma types induced different effects on DC maturation with oesophageal inducing DC activation, rectal inducing minor activation and colonic inducing inhibition of DC maturation markers. Interestingly, all cancer types induced DC inhibition of secreted TNF alpha. It was also found that 2Gy-irradiated TME induced significant inhibition of DC maturation for irradiated rectal adenocarcinoma and no effect with irradiated oesophageal cancer. Differential levels of inflammatory (IL2) and angiogenic mediators (Ang2 and bFGF) in TCM of GI tumours correlated with DC maturation.
Overall we found that there are differences in the human TME from different gastrointestinal cancers which can directly induce varying levels of inhibition of LPS-induced DC maturation.

P.B2.06.12

Investigation of the roles of anti-VEGFR1 natural antibodies in human plasma in hepatocellular carcinoma

C. Rodgers, *A. Pritchard, J. Wei;*
University of the Highlands & Islands, Inverness, United Kingdom.

Natural antibodies have been found to serve as an important anti-tumorigenic system in the body and their anti-tumor cytotoxicity has been confirmed with in vitro study. Natural antibodies are defined as the immunoglobulins produced by B1 lymphocytes in the absence of exogenous antigen stimulation. They are physiologically involved in maintaining tissue homeostasis such as clearance of apoptotic cell debris, elimination of invading pathogens as well as destruction of cancer cells formed in the body. In recent studies, it was found that natural IgG antibodies against vascular endothelial growth factor receptor 1 (VEGFR1) present in human plasma could be used to treat patients with hepatocellular carcinoma (HCC).
This study was thus designed to look at whether different epitopes derived from the VEGFR1 extracellular domain render HCC cells to have different responses to natural anti-VEGFR1 IgG in human plasma. An enzyme-linked immunosorbent assay was developed in-house with synthetic VEGFR1-derived peptides to screen human plasma rich in anti-VEGFR1 IgG that was applied to test the inhibitory effects of natural anti-VEGFR1 IgG on proliferation of HCC cell lines. Anticancer mechanism by which natural anti-VEGFR1 IgG kills HCC cells will be also investigated through analysis of cell viability, apoptosis, autophagy, gene expression, luciferase reporter assay, wound-healing and transwell migration.

P.B2.06.13

Alterations of the immune environment of Sezary cells

M. Roelens[1], *C. Ram Wolff*[2], *M. Delord*[3], *G. Maki*[4], *A. Marie-Cardine*[5], *A. Bensussan*[6], *M. Bagot*[6], *A. Toubert*[1], *H. Moins-Teisserenc*[1];
[1]*INSERM1160, IUH, AP-HP, Paris, France,* [2]*AP-HP, Hopital Saint Louis, Service de Dermatologie, Paris, France,* [3]*Biostatistics and Clinical Epidemiology, INSERM UMR-1153, Paris, France,* [4]*AP-HP, Hopital Saint Louis, Laboratoire d'Immunologie-Histocompatibilité, Paris, France,* [5]*INSERM UMR-976, UFR de Medecine, Paris, France,* [6]*INSERM UMR-976, UFR de Médecine, Paris, France.*

Introduction Sézary syndrome (SS) is a leukemic and aggressive form of Cutaneous T-cell Lymphomas. A challenge in SS is the preservation of antitumor and anti-infectious immunities, as infections are the most frequent cause of lethality. We previously defined the CD158k/KIR3DL2 molecule as a positive "generic" cell-surface marker for Sézary cells (SCs), and found an unexpected heterogeneity of naive/memory subsets. Such marker allows the characterization of non-SCs. Our aim was to analyze the phenotypes and functions of the "benign CD4+ T-cell counterpart" as well as other components of the innate and the adaptive immunity in the context of SS. **Methods**: Blood samples from patients and skin biopsies collected the same day, were analyzed using flow-cytometry for T-cell differentiation markers, resident-memory markers, as well as skin-homing, interleukins receptors and immune-checkpoint molecules. TCR repertoire was analyzed for 3 patients. Patients' derived monocyte subsets were analyzed both phenotypically and after maturation and differentiation into dendritic cells in proliferation assays.**Results**: We show that the "benign CD4+ T-cell counterpart" of SCs shares phenotypic similarity with malignant cells. These cells display skewed phenotypes and repertoire with enhanced expression of exhaustion molecules and defects in proliferative capacities. These abnormalities are not confined to the CD4+ T-cell-subset, as monocytes show a significant downregulation of HLA-DR molecules.**Conclusion**: Our results are in favor of profound alterations of the immune environment of SCs at phenotypic and functional levels. We are currently examining the relationships between SCs and the non-malignant immune compartment in both blood and skin compartments.

P.B2.06.14

Hypoxia effect on macrophages activation and fibroblasts cross-talk in chronic inflammation and tumor microenvironment

E. Setten[1,2], *M. Locati*[1,2];
[1]*Humanitas Clinical and Research Center, Rozzano (MI), Italy,* [2]*Department of Medical Biotechnologies and Translational Medicine, Università degli Studi di Milano, Segrate (MI), Italy.*

Macrophages (Mϕ) are highly plastic cells, able to assume different functional phenotypes depending on the microenvironment. To investigate the relevance of different microenvironmental cues on Mϕ activation, we generated in vitro Mϕ with an inflammatory phenotype using LPS+IFNγ (M1) or with an anti-inflammatory and pro-tumoral functional profile using IL4 (M2). In inflamed tissues and tumor lesions macrophages accumulate in areas of hypoxia, which is known to impact on macrophages activation. Similarly, in these contexts the interplay of macrophages with non-immune cells, such as fibroblasts, likely affects their biology. This project was planned in order to define the relative relevance of these cues and the underlying molecular mechanisms for the cross-talk between Mϕ and fibroblasts. Hypoxia-responsive genes (VEGF-A, GLUT1, MMP7, CXCR4) have been investigated by qRT-PCR in resting and activated Mϕ at short and extended time-points under normoxic or hypoxic conditions. We observed that Mϕ respond to hypoxia inducing specific transcripts and that their polarization has no significant impact on the induction of genes strictly related to hypoxia. On the contrary, hypoxia interferes with the expression of M1 transcripts, while having no impact on regulation of M2 genes. Model parameters will be extracted from molecular profiling approaches investigating Mϕ polarized to M1 and M2 settings under normal and hypoxic conditions in single cell cultures and direct-contact co-cultures with fibroblasts to better clarify the role of Mϕ in chronic inflammation and in tumor biology and to translate candidate genes into predictive biomarkers to support clinical decisions in therapy.

P.B2.06.15

Targeting PD-1/PD-L1 Pathway in tumor Microenvironment of Sebaceous Gland Carcinoma as a New Paradigm of Immunotherapy

L. SIngh[1], *M. K. Singh*[2], *S. Kashyap*[2], *S. Sen*[2], *M. A. Rizvi*[1];
[1]*Jamia Millia Islamia, New Delhi, India,* [2]*All India Institute of Medical Sciences, New Delhi, India.*

Introduction: Sebaceous gland carcinoma (SGC) is a malignant eyelid cancer and exhibit aggressive behaviour as it metastasizes to lymph nodes and distant organs. Understanding tumor-stromal interaction in tumor microenvironment has led to the development of immunotherapy in SGC. Programmed cell death-1/ligand (PD-1/PD-L1) interaction negatively regulates T cell activity and helps in the tumor escape mechanism. The aim of this study was to analyze PD-1/PD-L1 expression in tumor microenvironment as a prognostic marker and as a possible therapeutic target for SGC. Methods: Expression of immune markers (PD-1 and PD-L1 protein) was evaluated in 52 prospective cases of sebaceous gland carcinoma by immunohistochemistry and qRT-PCR. Results were correlated with clinicopathological parameters and patient outcome by statistical analysis. Results: Histopathological analysis revealed that 19 (36.5%) tumours were poorly differentiated and pagetoid spread was present in 22 cases. Immunoreactivity of PD-1 and PD-L1 expression was found in tumor and stroma cells. tPD-L1 showed immunoexpression in 51.92% cases whereas tPD-1 showed positive expression in 34.61%. sPD-1+ was more common than sPD-L1+ expression.

On univariate analysis, pagetoid spread, lymph node metastasis and tumor expressing PD-L1 expression was associated with a reduced disease-free survival (P<0.005). However, on multivariate analysis, only the tPD-L1 was associated with poor prognosis (P = 0.01). Conclusion: This is the first of its kind study, which states the role of tumor microenvironment in mediating the immune response in the pathogenesis of SGC. This paves the way for development of immunotherapy as a new strategy for treatment of metastatic sebaceous gland carcinoma patients.

P.B2.06.16

Interaction of NFκB-1 protein Expression with Inflammatory Microenvironment of Uveal Melanoma and its Prognostic Significance

M. K. Singh[1], *S. Kashyap*[2], *N. Pushker*[1], *S. Sen*[1], *R. Meel*[1], *K. Chodsol*[1], *S. Bakhshi*[1], *J. Kaur*[1];
[1]*AIIMS, New delhi, India,* [2]*AIIMS, New Delhi, India.*

Introduction: Uveal melanomas as malignant phenotype having a high density of macrophages, blood vessels and T-lymphocytes along with the presence of epithelioid cells and high melanin pigmentation which might be related to worse prognosis. High densities of inflammatory cells in uveal melanoma are associated with poor prognostic factors. NFκB-1 plays an important role in inflammation which promote cancer initiation and progression. The aim of the study is to detect NFκB-1 expression in the inflammatory microenvironment of uveal melanoma and its prognostic significance. **Method:** Evaluation of NFκB-1 expression was assessed by using immunohistochemistry and western blotting in 75 formalin fixed uveal melanoma tissues and transcriptional analysis was done on 58 fresh frozen tissues by real time pcr. Results were then correlated with clinicopathological parameters. **Results:** Out of 75 cases, 40 cases showed both nuclear and cytoplasmic expression and 15 showed cytoplasmic only. qRT-PCR showed upregulation of NFκB-1gene in 72.41% cases at transcriptional level. Expression of both cytoplasmic and nuclear c-REL protein showed significant correlation with cases having high tumour infiltrated lymphocytes, macrophages (CD68+) and presence of blood vessels (CD34+). There was a statistically significant difference in the overall survival of patients with nuclear/cytoplasmic NFκB-1 immunopositivity (p<0.05). **Conclusion:** This preliminary data suggests that NFκB-1 protein might play a role in the inflammatory microenvironment of uveal melanoma which is responsible for the pathogenesis of this disease. Further translational studies are required to explore the nature of NFκB-1 interactions in tumour microenvironment of uveal melanoma.

P.B2.06.17

Regulation of the expression of IL-1R8, a regulatory member of the Interleukin-1 receptor family

D. Supino[1], *C. Perucchini*[1], *M. Molgora*[1], *A. Ponzetta*[1], *S. Di Marco*[1], *E. Magrini*[1], *S. Carnevale*[1], *F. Gianni*[1], *S. Jaillon*[1,2], *A. Mantovani*[1,2], *C. Garlanda*[1,2];
[1]*Humanitas Clinical and Research Center, Rozzano, Italy,* [2]*Humanitas University, Rozzano, Italy.*

IL-1R8 is an Interleukin-1 receptor (ILR) family member which activates an anti-inflammatory program by inhibiting ILR and Toll like receptor (TLR) signaling. In NK cells IL-1R8 acts as checkpoint molecule in cancer and viral infections. The purpose of this study was to dissect the regulation of IL-1R8 expression in leukocytes. ChIP-seq analysis suggested that Colony Stimulating Factors (CSF)-inducible Transcription Factors and epigenetic modifications affect IL-*1R8* in NK cells and macrophages along maturation and activation. *IL-1R8* expression was down-regulated during macrophage differentiation. Pro-inflammatory molecules involved in macrophage and NK cell activation down-regulated *IL-1R8* expression in human and mice. Prostaglandin E2 and Interleukin-10 (IL-10), which are involved in cancer-associated immunosuppression, counteracted this effect in NK cells and up-regulated IL-1R8 in human monocytes and macrophages. *RNA*-seq analysis, q-PCR and Western Blot also revealed the existence of IL-1R8 truncated forms in human immune cells constituted by the exons coding for the intracellular part of IL-1R8, after M1 polarization. Thus pro-inflammatory stimuli are implicated in conventional isoform IL-1R8 down-regulation, overexpression of truncated forms of the protein, whose biological role is presently unknown. In addition, the up-regulation of IL-1R8 in NK cells by PGE2/IL-10 axis suggests that IL-1R8 could be part of the immunosuppressive activity of these molecules. IL-1R8 acts as a checkpoint molecule tuning antitumor and antiviral NK cell activity. Understanding how IL-1R8 expression in NK cells is affected by the tumor microenvironment is essential in the development of this checkpoint as a potential immunotherapy target.

P.B2.06.18

Patterns of immune checkpoint expression by primary tumor cells and tumor infiltrating lymphocytes across different tumor entities

M. Thelen[1], *A. Lechner*[1,2], *K. Wennhold*[1], *D. Pfister*[3], *F. Dörr*[4], *M. Heldwein*[4], *K. Hekmat*[4], *D. Beutner*[5], *M. Mallmann*[6], *F. Thangarajah*[6], *C. Bruns*[7], *M. von Bergwelt-Baildon*[1,8,9], *H. A. Schlößer*[1,7];
[1]*Cologne Center for Molecular Medicine, Cologne, Germany,* [2]*Department of Head and Neck Surgery, LMU, Munich, Germany,* [3]*Department of Urology, University of Cologne, Cologne, Germany,* [4]*Department of Cardiac and Thoracic Surgery, University of Cologne, Cologne, Germany,* [5]*Department of Head and Neck Surgery, University of Göttingen, Göttingen, Germany,* [6]*Department of Gynecology Surgery, University of Cologne, Cologne, Germany,* [7]*Department of General, Visceral and Cancer Surgery, University of Cologne, Cologne, Germany,* [8]*German Cancer Consortium (DKTK), Heidelberg, Germany,* [9]*Department of Internal Medicine III, LMU, Munich, Germany.*

Immune-checkpoint inhibition (CKI) demonstrated breakthrough therapeutic efficacy in several kinds of cancer. These therapies are unique, as the primary target is not the tumor cell itself, but the crosstalk between immune cells and cancer cells in the tumor microenvironment. Efficacy of CKI is not limited to patients with expression of the respective protein on tumor cells and recent publications demonstrated that expression of PD-L1 on tumor-infiltrating lymphocytes (TIL) can be of similar importance.
Expression patterns of 30 described immune checkpoint and regulatory molecules were analyzed on T, B and NK cells in peripheral blood and single cell suspensions of primary tumor samples of nine different tumor entities using flow cytometry. Expression of the respective ligands on primary tumor cells was assessed in tissue microarrays.
The majority of analyzed immune checkpoint pathways could be detected. Despite the variety of primary tumor sites, our analyses revealed similar expression patterns for most molecules included in this study. For example, PD-1 as well as PD-L1 were detectable on tumor cells and tumor-infiltrating lymphocytes in all analyzed tumor entities and expression patterns on TIL were largely overlapping. In addition, we correlated immune checkpoint expression to the infiltration by lymphocytic subsets including regulatory T cells.
Immune escape is a common feature of cancer and the specific expression patterns described in this study are of translational relevance for ongoing and future immunotherapeutic trials.

P.B2.06.19

Potential γδ T cell transdifferentiation into αβ T cells in transplanted children

A. Zorzoli[1], *G. Barbarito*[2], *P. Merli*[3], *F. Antonini*[4], *A. Bertaina*[2], *E. Ferretti*[1], *F. Frassoni*[1], *F. Locatelli*[3], *I. Airoldi*[1];
[1]*Laboratorio Cellule Staminali post natali e Terapie Cellulari, Istituto Giannina Gaslini, Genova, Italy,* [2]*Pediatrics Stem Cell Transplantation, Stanford University, Palo Alto, United States,* [3]*Dipartimento di Onco-Ematologia e Terapia Cellulare e Genica, Ospedale Pediatrico Bambino Gesù, Roma, Italy,* [4]*Core Facilities, Istituto Giannina Gaslini, Genova, Italy.*

We recently demonstrated that pediatric patients with acute leukemia receiving a graft depleted of αβ T and CD19+ B lymphocytes and treated with zoledronic acid (ZOL) showed: i) increased cytotoxicity of γδ T cells against leukemic blasts, ii) rapid reconstitution of αβ T cells and iii) a decrease of GvHD incidence. These data suggested that γδ T cells, reconstituted or infused with the graft, could induce αβ T cell reconstitution.
In this contest, it has been reported that the specific population of Vδ1+CD4+ γδ T cells can trans-differentiate into αβ T cells, through specific steps of TCR rearrangement.
Thus, we hypothesized that ZOL could be an appropriate stimulus for the process of trans-differentiation of γδ T into αβ T cells in transplanted patients.
In order to validate this hypotesis we started a set of experiments in order to characterized T cells from transplanted patients infused and not with ZOL.
Furthermore, we tested whether human γδ T cells may transdifferentiate in αβ T cell using highly immunodeficient mice NOD/SCID/IL2rg-/- (NSG).
Preliminary results showed that in transplanted patients treated with ZOL the population of Vδ1+CD4+ γδ T cells appears, and that NSG mice may represent a suitable model to recapitulate trans-differentiation of γδ T into αβ cells.
Further investigations are needed to confirm that the trans-differentiation of γδ T into αβ cells may be an innovative immunotherapy against pediatric acute leukemia.
GRANT: A.I.R.C. IG 17047

P.B2.06.20

CD117 is expressed following CD8⁺ T cell priming and stratifies sensitivity to apoptosis according to strength of initial engagement

G. Frumento, ***J. Zuo****, K. Verma, P. Moss;*
Insititute of Immunology and Immunotherapy, Birmingham, United Kingdom.

CD117 (cKit) is the receptor for stem cell factor (SCF) and plays an important role in the development of early thymocytes, but it was not known to be expressed downstream the triple negative stage. Since we found CD117 transcripts in activated mature T cells, we studied the characteristics of the phenomenon. We found that CD117 is expressed following priming of mature CD8⁺ naive T cells *in vitro* and is detectable *in vivo* in CD8⁺ T cells following primary Epstein-Barr virus infection. CD117 expression is mediated through an intrinsic pathway and is suppressed by IL-12. Importantly, the extent of CD117 expression is inversely related to the strength of the activating stimulus and subsequent engagement with cell-bound SCF markedly increases susceptibility to apoptosis. CD117 is therefore likely to shape the pattern of CD8⁺ T cell immunodominance during a primary immune response by rendering cells with low avidity for antigen more prone to apoptosis. Furthermore, CD117⁺ T cells are highly sensitive to apoptosis mediated by galectin-1, a molecule commonly expressed within the tumour microenvironment, and expression may therefore represent a novel, and potentially targetable, mechanism of tumour immune evasion.

P.B2.07 Environmental regulation anti-tumor responses - Part 7

P.B2.07.01

Promoting NK cells anti-viral and anti-tumor function by *in vitro* manipulation

E. Badami[1], *F. Barbera*[2], *A. Gallo*[2], *C. Coronnello*[1], *D. Paini*[1], *P. Conaldi*[2];
[1]*Fondazione RiMED, Palermo, Italy,* [2]*ISMETT, Palermo, Italy.*

Background/Aims. Natural Killer (NK) cells respond to infection and tumor by releasing pro-inflammatory cytokines (IFNγ, TNFα) or by cell-to-cell contact using TRAIL-mediated apoptosis. Aim of this study was to discover if response to viral infection or cancer cells can be improved by manipulating NK cells *in vitro* with selected cytokines.
Methods. Healthy donors NK cells were differentiated with a selected mix of cytokines into cytotoxic TRAIL$^+$ or cytokine-releasing IFNγ$^+$TNFα$^+$ NK cells and phenotype assessed by Flow Cytometry. Cytotoxicity was determined by CRA. NK immune function was studied in transwell co-cultures with target cells +/- HCV; miRNome signature was investigated by NGS and cytokine profiling by Multiplex.
Results. NK cell differentiation with any of the cytokine cocktails tested induced upregulation of NKG2D, NKp30, NKp44 and NKp46. By cell-cell contact, cytotoxic TRAIL$^+$NK cells killed with the highest efficiency target cells, while cytokine-releasing IFNγ$^+$TNFα$^+$ NK were less functional. By transwell co-culture, we observed that TRAIL$^+$NK cells fully eradicated HCV infection and reduced the tumor phenotype by releasing soluble factors, while IFNγ$^+$TNFα$^+$NK were less efficient. By comparative miRNome analysis we underpinned a number of novel miRNA. Notably, hierarchical clustering showed systematic variations in the miRNA expression among the different groups. Protein analysis produced pattern of soluble factors and will be here discussed.
Conclusions. The pathways identified in activated TRAIL$^+$ NK cells that specifically characterize their enhanced anti-viral and anti-tumor function compared to IFNγ$^+$TNFα$^+$NK described herein might represent a tool to license fully functional NK cells with translational potential for clinical applications.

P.B2.07.02

Underlying mechanisms of tumor recurrence after incomplete cancer immunotherapy

E. Beyranvand Nejad*, C. Labrie, S. van Duikeren, T. van Hall, R. Arens, S. H. van der Burg;*
Leiden University Medical Center, Leiden, Netherlands.

Cancer vaccines aim to induce specific T cell responses directed against tumor cells. Previously, we have shown therapeutic efficacy of vaccination with synthetic long peptide (SLP) vaccines in mouse tumor models and in patients with human papilloma virus-induced neoplastic lesions. However, under less optimal vaccine conditions the SLP-vaccinated mice display tumor recurrences despite the initially T-cell mediated regression induced by the vaccine. Here, we investigated the underlying mechanisms focusing on the tumor microenvironment. Our data showed that recurrent tumors displayed a significantly reduced leukocyte infiltration in particular CD8 T cells compared to primary tumors. This was accompanied by lower levels of chemokine receptors including CXCR3 on the T cells, and a reduced chemokines production by intratumoral immune cells. Moreover, although these T cells are capable of producing inflammatory cytokines, the cytotoxic capacity to kill tumor cells was inferior compared to the T cells at the time of regression. Notably, we did not observe any difference in the expression of T cell inhibitory molecules such as PD-1, Tim3 and LAG3 at the time of relapse, confirming our results that blocking PD-1 did not prevent the tumor recurrence. Interestingly, vaccination of mice that were injected with recurrent tumor cells did not result in tumor regression. Together, that the observed defective T cell infiltration and increasing intrinsic tumor resistance to killing occurs suggests that incomplete cancer immunotherapy leads to immune selection and tumor escape. On-going work exploring immune selection and tumor heterogeneity by using RNA seq analysis and cell barcoding will be discussed.

P.B2.07.03

A regulatory macrophage phenotype induced by IgG4: implications for tumour-mediated immune tolerance

R. Bianchini[1,2], *S. A. Jensen*[1,3], *G. Jordakieva*[2,4], *A. Groschopf*[1,2,5], *J. Piehslinger*[6], *D. Reichhold*[6], *A. Klaus*[6], ***E. Jensen-Jarolim***[1,2];
[1]*The Interuniversity Messerli Research Institute, University of Veterinary Medicine Vienna, Medical University of Vienna, Dept. of Comparative Medicine, Vienna, Austria,* [2]*Institute Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Division of Comparative Immunology and Oncology, Medical University of Vienna, Vienna, Austria,* [3]*Department of General Surgery, Medical University of Vienna, Vienna, Austria,* [4]*Department of Physical Medicine, Rehabilitation and Occupational Medicine, Vienna, Austria,* [5]*FH Campus Wien, University of Applied Sciences, Vienna, Austria,* [6]*Department for Surgery, Hospital "Barmherzige Schwestern", Vienna, Austria.*

Background: Tumour is associated with the M2 microenvironment as well as M2a-like macrophage accumulation. During allergen-specific immunotherapy (AIT) that M2 microenvironment is modulated by T-reg and B-reg cells via secreting IL10 resulting in class switch from IgE to IgG4. Moreover, IgG4 has also been found in tumour tissues correlated with poor clinical outcome. We hypothesized that tumour tissue IgG4 may interact with M2a macrophages and drive them into an immunoregulatory phenotype.
Methods: We analysed plasma levels of total IgE, IgG1 and IgG4 from healthy controls and non-metastatic or metastatic colon cancer patients. Moreover, we checked if monocyte derived macrophages from different donors would respond differently in presence of IgG1 or IgG4. **Results:** A significantly higher IgG4/IgE plasma level ratio was revealed in metastatic tumour patients compared to the other groups (p=0.025 and p=0.014 vs Healthy and non-metastatic tumour donors). This was associated with a lower surface marker expression of CD206, CD163 and CD86 and higher cytokine production of CCL1, IL10 and IL6 in IgG4-stimulated M2a macrophages in all groups, compared to IgG1 or no IgG stimulation. **Conclusions:** Our data indicate that macrophages from tumour patients do not differ in their capacity of polarization in comparison with healthy donors. However, stimulation with IgG4 may drive M2a macrophages to an immunoregulatory M2b-like phenotype. Therefore, the presence of IgG4 in tumour tissue repolarizes tumour-associated macrophages and both synergize in shaping a strong immunosuppressive microenvironment decisive for a poor prognostic outcome. **Supported by FWF-project SFB F4606-B28 to EJJ.**

P.B2.07.04

Murine acute myeloid leukemia alters NK cells maturation and functions by affecting IL-15 signaling

B. Bou Tayeh[1], *V. Laletin*[1], *J. Fares*[1], *R. Leblanc*[1], *D. Payet-Bornet*[2], *B. Nadel*[1], *S. JC Mancini*[1], *O. Herault*[3], *D. Olive*[1], *M. Aurrand-Lions*[1], *C. Fauriat*[1];
[1]*Centre de Recherche en Cancérologie de Marseille (CRCM), INSERM, CNRS, Institut Paoli-Calmettes, Aix-Marseille University, UM 105, Marseille, France,* [2]*Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université UM2, Marseille, France,* [3]*CNRS UMR 7292, LNOx Team, François Rabelais University, Tours, France.*

The immune system is ineffective against tumor progression in acute myeloid leukemia (AML). Among immune effectors with anti-leukemic activity, Natural Killer (NK) cells display altered phenotype, functions, as well as molecular alterations at the diagnosis of leukemic patients. Although it has become clear that leukemic cells are responsible for these alterations, the molecular mechanisms by which they subvert NK cell functions remain elusive. Here, we have used syngeneic mouse model for AML to decipher these mechanisms. Hence, as AML progressed in mice, we observed a selective enrichment of CD27$^+$CD11b$^-$ immature NK cells. Additionally, AML progression resulted in the decrease of NK cell degranulation and cytokine production, irrespective of development stages.
Moreover, it has been shown that IL-15 signaling controls both maturation and activation of NK cells, two checkpoints that are altered in our model. Hence, we studied the effect of *in vitro* and *in vivo* activation of IL-15 signaling in leukemic mice NK cells. We noted lower phosphorylation of essential components of IL-15 signaling, which was associated with lower expression of IL-15 receptors leading to a decreased nutrient uptake and NK cell growth. Additionally, molecular analysis of murine AML cells showed expression of TGF-β, an immunosuppressive cytokine capable of inhibiting IL-15 signaling.
We propose that AML cells may alter NK cell functions and maturation by secreting TGF-β. Our preliminary results are in line with observations made in human patients and will be determinant to better understand the mechanisms of NK cells dependent immune evasion in AML.

P.B2.07.05

Red pulp macrophages in the spleen provide a niche for chronic myeloid leukemia stem cells

E. D. Bührer[1], *M. A. Amrein*[1], *S. Isringhausen*[2], *C. Nombela-Arrieta*[2], *C. Schürch*[3], *S. Bhate*[3], *R. Radpour*[1], *C. Riether*[1], *A. F. Ochsenbein*[4];
[1]*Department of BioMedical Research, Bern, Switzerland,* [2]*Department of Hematology, Zürich, Switzerland,* [3]*Baxter laboratory for stem cell biology, Stanford, United States,* [4]*Department of Medical Oncology, Bern, Switzerland.*

Chronic myeloid leukemia (CML) is a malignant myeloproliferative disorder characterized by the constitutively active tyrosine kinase BCR/ABL1. Disease progression and relapse is caused by therapy resistant leukemia stem cells (LSCs), and cure relies on their eradication. The microenvironment in the bone marrow (BM) has been extensively studied and is known to contribute to LSC maintenance and resistance. Leukemic infiltration of the spleen is a hallmark of CML. However, the detailed composition of the splenic niche in CML and how it affects and maintains LSCs is unknown. In a mouse model of CML, we demonstrated that primitive leukemic stem and progenitor cells (LSPCs) preferentially accumulated in the spleen and contributed to disease progression by increasing LSC numbers in the BM. RNA sequencing of spleen and BM LSPCs revealed enriched stemness and decreased myeloid lineage priming in LSPCs of the spleen. Moreover, LSCs in the spleen were more quiescent than in the BM and showed increased resistance to tyrosine kinase inhibitor (TKI) therapy. Furthermore, spleen LSCs were exclusively located in the red pulp. Depletion of macrophages by clodronate treatment reduced LSC numbers in the spleen, whereas LSCs in the BM were not affected. In spleens of human CML patients, LSCs co-localized with red pulp macrophages (RPMs). These results reveal the spleen as an independent, disease-promoting niche for primitive LSPCs. Thus, targeting the splenic niche may be necessary to eradicate LSCs and cure CML.

P.B2.07.06

MHC class I modulation by iron metabolism and NK cells recognition

E. Carbone[1,2], *R. Sottile*[3,4], *G. Federico*[5], *C. Garofalo*[1], *R. Tallerico*[1], *C. Faniello*[6], *B. Quaresima*[6], *C. Cristiani*[1], *G. Cuda*[6], *V. Ventura*[1,7], *N. Perrotti*[8], *S. Ferrone*[9], *E. Gulletta*[10], *K. Kärre*[4], *F. Carlomagno*[5];

[1]*Tumor Immunology and Immunopathology Laboratory, Department of Experimental and Clinical Medicine, University "Magna Græcia" of Catanzaro, Catanzaro, Italy, catanzaro, Italy,* [2]*Department of Microbiology, Cell and Tumor Biology (MTC), Karolinska Institutet, Stockholm, Sweden, Stockholm, Sweden,* [3]*Tumor Immunology and Immunopathology Laboratory, Department of Experimental and Clinical Medicine, University "Magna Græcia" of Catanzaro, Catanzaro, Italy, Catanzaro, Italy,* [4]*Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, 17177, Stockholm, Sweden., Stockholm, Sweden,* [5]*Department of Molecular Medicine and Medical Biotechnologies Federico II University, Naples, Italy, Napoli, Italy,* [6]*Centro di Ricerca di Biochimica e Biologia Molecolare Avanzata, Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi "Magna Græcia", Catanzaro, Italy; Dipartimento di Medicina Sperimentale e Clinica, catanzaro, Italy,* [7]*Department of Health Sciences, University "Magna Græcia" of Catanzaro, Catanzaro, Italy, Catanzaro, Italy,* [8]*Unit of Medical Genetics, "Mater Domini" University Hospital, Catanzaro, Italy, catanzaro, Italy,* [9]*Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA, Boston, United States,* [10]*Department of Health Sciences, University "Magna Græcia" of Catanzaro, Catanzaro, Italy, catanzaro, Italy.*

Introduction: The iron concentration in the environment is crucial element for both pathogens and innate immunity. We investigate whether extracellular iron concentrations and intracellular ferritin heavy chain (FHC) may modulate MHC expression and NK cells cytotoxicity. This new and interesting link between iron metabolism and immunity can be exploited in the treatment of infections and cancer.
Materials and Methods: Primary melanoma tumor cells were treated with DFO and IFN gamma and established tumor cell lines were silenced with shFHC vector. PBMC derived from hemocromathosis patients' blood were isolated. All cell system were analysed for the expression of MHC molecules and NK cytotoxicity assays. To analyze ex vivo and in vivo the relationship between FHC annd MHC class I experresion the NCO4 -/- mice was used.
Results: FHC downregulation, either by environmental iron chelation or shRNA transfection, led to MHC-class I surface reduction. Moreover, low concentrations of iron in microenvironment interfere with IFN gamma receptor signaling preventing the related increase of MHC-class I molecules expression. Furthermore, ex vivo evidences confirm the in vitro observations: a) mouse bearing a NCO4-/- gene deletion leads to FHC accumulation and MHC class I cell surface overexpression b) Hemochromatosis patients with high iron blood concentrations expresses higher levels of MHC-I on their cell surface. Tumor cells growth in low iron environment are highly sucsceptible to NK cytotoxicity.
Conclusions: We propose a role for H-ferritin and iron metabolism in regulating the MHC class I expression in humans and mice and the related NK susceptibility.

P.B2.07.07

Blocking of beta adrenergic signalling improves the efficacy of anti-tumor responses

C. Daher*, L. Vimeux, E. Peranzoni, R. Stoeva, E. Donnadieu, A. Trautmann, N. Bercovici, V. Feuillet;*
Institut Cochin, Inserm U1016 CNRS UMR 8104, Université Paris Descartes, Paris, France.

Background: Adrenergic signals are known to exert a major influence in cancer. In addition to a direct action on tumor growth, numerous arguments support potential effects on anti-tumor immune responses. Our goal was to characterize the effects of adrenergic signals on anti-tumor responses, and to estimate the therapeutic interest of β–blockers for improving them.
Material and methods: To address this question, we used two murine tumor models: 1) a model of vaccine-induced regression of transplanted tumors (TC1) and 2) a model of progressing spontaneous mammary tumors (MMTV-PyMT) in which the "natural" anti-tumor response is in check. In both models, we evaluated the effects of a chronic treatment with propranolol (a β–blocker) by using multicolour flow cytometry, gene expression and imaging.
Results: In TC1 model, propranolol strongly improved the vaccine efficiency by increasing the number of $CD8^+$ T cells infiltrating the tumor. Moreover, we demonstrate that this effect mainly occurred during the priming of T cells in the draining lymph node (Dr-LN). In MMTV-PyMT tumors, tumor growth was markedly slowed down by propranolol, which also increased tumor infiltration by $CD8^+$ T cells. Finally, in vitro, adrenergic signalling had a major inhibitory effect on T cell activation, a phenomenon likely to alter T cell reactivity in both Dr-LN and the tumor.
Conclusions: Our results allow a better understanding of the influence of adrenergic signalling on anti-tumor responses. They provide a basis for the strategic use of β-blockers to improve them.
This work is supported by the Ligue Contre le Cancer IDF.

P.B2.07.08

Multiparameter flow cytometry immunophenotypic identification and characterization of tumor infiltrating immune cells in glioblastoma multiforme

M. González-Tablas[1,2], *Á. Otero*[2,3], *D. Pascual*[2,3], *L. Ruiz*[2,3], *D. Miranda*[2,3], *P. Sousa*[2,3], *D. Arandia*[2,3], *M. Jaramillo*[2,3], *J. Gonçalves*[2,3], *A. Orfao*[1,2], *M. Tabernero*[1,2,4];
[1]*Centro de Investigación del Cáncer (CIC-IBMCC; CSIC/USAL; IBSAL) y Departamento de Medicina Universidad de Salamanca, Salamanca, Spain,* [2]*Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, Spain,* [3]*Servicio de Neurocirugía, Hospital Universitario de Salamanca, Salamanca, Spain,* [4]*Instituto de Estudios de Ciencias de la Salud de Castilla y León (IECSCYL), Soria, Spain.*

Glioblastoma multiforme (GBM) is the most common and aggressive adult primary tumor of the brain. The specific distribution and role of tumor immune cells in favoring vs blocking malignant transformation, tumor progression and growth, remains unknown. Here we characterize the cellular composition of tumor immune infiltrates from primary GBM by multiparametric flow cytometry.
Resected primary tumors from 39 adults diagnosed with GBM were analyzed. Single tumor cell suspensions were obtained by mechanical disaggregation and stained with 8-color monoclonal antibody combinations for the enumeration of tumor infiltrating myeloid (CD45, HLADR, CD14, CD11B, CD16, CD15, CD33, CD192 and CD206) and lymphoid cells (CD3, CD4, CD8, CD19, CD20, CD25 and CD127). Stained samples were measured in a Fortessa X20 flow cytometer and analyzed with the Infinicyt software.
Overall, tumor infiltrating immune cells (TIICs) represented (median) 27% of the whole cellularity, with highly-variable numbers among distinct tumors (range: 3%-73%). Myeloid cells with a monocytic/dendritic cell phenotype predominated (23%; range: 2%-65%) with a minor proportion of neutrophils (2%; range: 0.2%-45%) and lymphoid cells (1.4%; range: 0.04%-8%). Among lymphocytes, CD8+ (0.5%; range: 0.02%-3%) and CD4+ T-cells (0.4%; range: 0.01%-3%) were represented at similarly low values, and extremely low numbers of regulatory T-cells (median: 0.05%) and B-lymphocytes (0.02%) were also found.
We demonstrate the feasibility of evaluating TIIC by flow cytometry and their systematic presence in GBM tumor samples at highly-variable levels, with clear predominance of antigen-presenting myeloid cells, over neutrophil and lymphoid infiltrates, their role in modulating the tumor microenvironment, deserves further investigations. (ISCIII ref PI16/0476)

P.B2.07.09

MHC II-dependent activation of regulatory T cells in the bone marrow of leukemia mice leads to immune evasion and disease progression

M. Hinterbrandner*, A. Ochsenbein, C. Riether;*
Bern University Hospital and Department for BioMedical Reasearch, University of Bern, Bern, Switzerland.

Leukemia stem cells (LSCs) in the bone marrow (BM) are the origin of leukemia and resistant against conventional therapies and immune control. This resistance is partially mediated by protective mechanisms of the hematopoietic stem cell niche in the BM. In leukemia, the BM microenvironment changes dramatically with regulatory T cells (Tregs) accumulating. However, little is known how Tregs affect LSCs.
We induced chronic myeloid leukemia (CML) in a murine model with BL/6 BCR-ABL-1 transduced LSKs (lineage$^-$Sca-1$^+$c-kit$^+$) in FoxP3$^{DTR\text{-}GFP}$ mice. We investigated the frequency, origin, activation and proliferation capacity of BM Tregs in CML compared to naïve mice and analyzed the Treg-accumulation during disease progression.
BM Tregs in CML mice were mostly thymic-derived, activated and showed higher proliferation capacity compared to controls. Treg-depletion resulted in long-term survival in the majority of the mice. Importantly, Treg-depleted CML mice showed decreased LSC numbers compared to controls phenotypically (FACS-analysis) and functionally by colony forming assays and secondary transplantation experiments. To show a direct Treg-LSC interaction or an indirect via CD8 T cells, we depleted Tregs and CD8 T cells simultaneously. Parallel depletion restored LSC numbers, suggesting that Tregs protect LSCs from CD8-mediated elimination. To investigate the activation of Tregs, we induced CML derived from MHC-II-deficient LSCs since we observed high MHC II expression on LSCs. MHC II$^{-/-}$ CML developed significantly slower than control CML and showed the same phenotype as the Treg-depleted CML mice.
Our data indicate that thymic-derived, MHC-activated Tregs protect LSCs from elimination by cytotoxic CD8 T cells and promote leukemia development.

P.B2.07.10

Cytomegalovirus-related cytokines improve natural killer cell activity against cancer cells

K. A. Holder[1], *J. Lajoie*[2], *M. D. Grant*[1];
[1]*Memorial University, St. John's, Canada,* [2]*University of Manitoba, Winnipeg, Canada.*

Through an unknown mechanism, cytomegalovirus (CMV) focuses the initially diverse natural killer (NK) cell repertoire into functionally and phenotypically distinct subsets. Given the global prevalence of CMV infection, at least 2 billion people have an adapted pool from within which NK cells can be selectively recruited against virus-infected or aberrant cells. We investigated whether cytokines produced in CMV infection alter NK cell activity against transformed cell lines.
Supernatants from CMV-infected (CMVsn) fibroblasts caused a 40% and 32% rise in NK cytotoxicity (K562) and antibody-dependent cellular cytotoxicity (ADCC; anti-MHC I-coated C1R-B27), respectively, enhancing NK activity through NKG2C, NKG2D, NKp30, and NKp46. While CMVsn had elevated levels of IL-6, IL-8, IL-15, IFN-α2 and IFN-β, disrupting stimulation through IFN-α/β receptors alone fully prevented increased NK cell activity. The CMV-encoded hIL-10 homolog (cmvIL-10) also enhanced NK cytotoxicity and we probed NK activity against a variety of malignant cell lines to study the breadth of cytokine-induced NK cell activation. CMVsn or cmvIL-10 treatment robustly increased NK cytotoxicity against breast (SKBR-3), ovarian (SKOV-3), prostate (22Rv1) and colon (HT-29) cancers, and myeloid (U937) leukemia. Exogenous cmvIL-10 also induced potent TNF-α and IFN-γ responses by NK cells from CMV^{pos} donors against antibody-coated SKOV-3 cells.
Adapted NK cells potentially offer a rich substrate for enabling broad immune activity against malignancy and could contribute to new iterations of cancer immunotherapy.

P.B2.07.11

Studying TGF-ß1 activation via GARP in megakaryocytes and its potential involvement in myeloproliferative neoplasms.

S. Lecomte*;*
de Duve Institute, Brussels, Belgium.

Myeloproliferative neoplasms (MPN) represent clonal proliferation of pathological hematopoietic stem cells. MPN encompass chronic myeloid leukaemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). MPN subtypes differ in their potential to develop bone marrow fibrosis, with PMF exhibiting the highest and ET having the lowest risk of progression to bone marrow fibrosis. The precise mechanisms leading to increased deposition of bone marrow stromal fibers remain unclear. A growing body of evidences suggest that it is mediated by Transforming Growth Factor-beta 1 (TGF-ß1) released by proliferating megakaryocytes. TGF-ß1 is a well-known pro-fibrotic cytokine. It is secreted by many cell types as an inactive form, called latent TGF-ß1. However, very few cell types have been shown to produce the active form of the cytokine, via mechanisms that are cell-type specific. Regulatory T lymphocytes (Tregs) can activate TGF-ß1 via GARP, a transmembrane protein that is induced on the surface after T cell receptor stimulation. GARP is also expressed on megakaryocytes. We want to address the question whether megakaryocytes can also activate TGF-ß1 via GARP, in physiological conditions, but more importantly in the context of myeloproliferative neoplasms where this mechanism could contribute to bone marrow fibrosis.

P.B2.07.12

Receptor expression and cytotoxicity of primary human NK cells is impaired in obesity

W. Naujoks[1], *A. Hauffe*[1], *J. Spielmann*[1], *I. Bähr*[1], *D. Quandt*[1], *J. Harth*[2], *H. Kielstein*[1];
[1]*Department of Anatomy and Cell Biology, Faculty of Medicine, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany,* [2]*Transfusion Medicine Unit, University Hospital, Halle (Saale), Germany.*

Introduction: Overweight and obesity are growing epidemic health problems. Obesity, as a major risk factor for developing severe cancers, e.g. colorectal and postmenopausal breast cancer, is associated with alterations in NK cell functionality. In the early phase of cancer development, NK cells are the central active component of a host's immune system. To date the pathophysiological mechanisms between obesity and cancer remain unclear. Therefore, the present study aimed to investigate the relation between bodyweight and NK cell receptor expression as well as NK cell cytotoxicity against tumor cells in humans.
Methods: PBMCs were collected from buffy coats of healthy blood donors with different body mass indexes (normal-weight, overweight, obese) and NK cell specific parameters were analyzed by flow cytometry. The cytolytic activity of isolated NK cells against human colon and breast cancer cell lines was analyzed by using an impedance-based cytotoxicity assay.
Results: Primary human NK cells isolated from obese donors compared to normal-weight donors showed a significantly decreased cytolytic activity against colon cancer cells. Flow cytometric analysis revealed an altered expression pattern of activating and inhibitory NK cell receptors and adhesion molecules on NK cell subsets of overweight and obese individuals compared to the normal weight control group.
Conclusions: The decreased cytotoxic activity against colon tumor cells and the altered receptor expression pattern of NK cells from overweight and obese individuals indicate that the linkage of obesity and the known increased risk for colorectal cancer can be related to an impaired NK cell functionality.

P.B2.07.13

Conformational epitope PAINS-13 on the CD9 tetraspanin is expressed on clonal plasma cells in a subset of patients with monoclonal gammopathies.

A. Roncancio-Clavijo*, C. Martín-Martín, E. Rodríguez-Martín, P. Walo, J. Fernández-Velasco, M. Espiño, M. Blanchard, L. Villar, E. Roldán;*
Hospital Universitario Ramón y Cajal, Madrid, Spain.

Monoclonal gammopathies (MG) are characterized by clonal proliferation of plasma cells (PC). Several molecules have been implicated in the adhesive interactions between PC and the bone marrow (BM) microenvironment, particularly beta 1 integrin (CD29), which is sometimes associated with tetraspanins. We described that clonal PC from the majority of MG patients express a functionally conformational epitope (PAINS-13) of the tetraspanin CD9. The study included diagnosed MG patients (41 MM, 14 MGUS). BM aspirates were stained using an indirect immunofluorescence technique with unconjugated PAINS-13 mAb (Dr. Cabañas; CBM, Madrid) and rat anti-mouse IgG3-FITC. BM aspirates were also stained with conjugated CD38, CD56, CD19 (to detect normal and clonal PC) and CD29, CD49d, CD49f or CD9 mAb to study the expression of adhesion molecules and tetraspanins. Flow cytometric studies showed the presence of PAINS-13 epitope in 29 out of 55 (52.7%) MG patients. There was not significant differences between MM or MGUS patients. PAINS-13 expression did not correlate neither with the active form of CD29 (HUTS21+ cells) nor with the level of CD49f expression. *In vitro* experiments also demonstrate that PAINS-13 expression was not associated with induction of activation of CD29 by divalent cation Mn^{2+}: whereas the addition of 0.5 mM Mn^{2+} clearly increased binding of mAb HUTS21 to MG cells, binding of PAINS-13 mAb was not influenced by CD29 activation state. Our results demonstrate that clonal PC usually express the CD9 conformational epitope PAINS-13 which did not depend on the previous activation of CD29.

P.B2.07.14

Destabilized liposomes as carriers for doxorubicin and siRNA to target tumor associated macrophages in a humanized mouse melanoma model

J. Schupp[1], *M. Voigt*[2], *M. Helm*[2], *A. Tuettenberg*[1];
[1]*Dept. of Dermatology, Mainz, Germany,* [2]*Johannes Gutenberg University, Mainz, Germany.*

An established tumor did overcome the patient's immune system and is exploiting its immune suppression mediating mechanisms to sustain tumor growth and avoid rejection. Repolarizing tumor associated macrophages into immunostimulatory M1 macrophages is a promising strategy to flip the switch in the tumor microenvironment from immune suppression towards an immune reaction against the tumor. In order to achieve repolarization of macrophages it is necessary to have (i) efficient nano carriers to transport small molecules or siRNA into macrophages and (ii) to subsequently release the functional cargo in an adequate amount. We developed destabilized liposomes being sensitive to low pH levels and physiological temperatures. The chemotherapeutic Doxorubicin and siRNA are used as cargo. We perform release studies *in vitro* and *in vivo*.
To analyze cargo release we perform *in vitro* cultures of human monocyte-derived macrophages and human melanoma cells. Doxorubicin release inside the cell is quantified by using flow cytometry and confocal microscopy. siRNA mediated transfection is detected via qPCR. *In vivo* studies are carried out in a subcutaneous melanoma model of human melanoma cell lines in humanized NOD/SCID mice transgenic for HLA-A2. Spleen and tumor showed a distinct composition of immune cell infiltration in immunohistochemistry staining proving the model to be valid for our purpose. Our data indicate, that destabilized liposomes show faster release of doxorubicin compared to stable liposomes. Control liposomes loaded without cargo are non-toxic. Therefore, we use them as nano carrier in our model. This work is supported by the DFG (CRC1066).

P.B2.07.15

Dissecting the role of MS4A4A in the context of macrophages

R. Silva-Gomes[1,2,3], *M. Sironi*[3], *R. Porte*[4], *I. Mattiola*[5], *A. Fontanini*[3,6], *M. Stravalaci*[4], *M. Oliveira*[2], *M. Locati*[6], *B. Bottazzi*[3], *A. Mantovani*[4,3];
[1]*Graduate Program in Areas of Basic and Applied Biology (GABBA), ICBAS, University of Porto, Porto, Portugal,* [2]*INEB-Institute of Biomedical Engineering/Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Porto, Portugal,* [3]*Department of Inflammation and Immunology, Humanitas Clinical and Research Center, Rozzano, Italy,* [4]*Humanitas University, Rozzano, Italy,* [5]*Department of Microbiology and Infection Immunology, Charité - University Medical Centre Berlin, Berlin, Germany,* [6]*Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy.*

Monocytes and macrophages (MØs) are key components of several pathological conditions, undergoing profound functional reprogramming influenced by the microenvironment. Several markers have been reported to identify specific MØs subsets and activation profiles. Recently, we described the tetraspanin MS4A4A as part of the transcription signature of M2-MØs and tumor-associated MØs (TAMs). It was shown that MS4A4A is selectively expressed in tissue resident MØs, is upregulated during polarization towards an M2/M2-like phenotype, being also highly expressed in TAMs. The pattern recognition receptor Dectin-1 was identified as a molecular partner of MS4A4A. MS4A4A-deficient MØs present an impaired Dectin-1-dependent crosstalk with NK cells, leading to uncontrolled metastatic spreading. Yet, several aspects of MS4A4A immunobiology remain unclear.
Our goal is to better understand the functional consequences of MS4A4A interaction with Dectin-1 and other partners. We are taking advantage of genetic models to characterize MS4A4A expression in the mouse. A second line of work concerns the characterization of MS4A4A role in carcinogenesis and metastatic spreading, with several models being currently under investigation.
Dectin-1 recognizes β-glucans in *Aspergillus fumigatus* cell wall, having a key role in the infection context. Thus, we are characterizing the susceptibility of *ms4a4a-ko* mice to *A. fumigatus* infection, a major risk factor in immunocompromised patients.
A better understanding of MS4A4A expression and function in the context of macrophages will pave the way to the use of this molecule as a therapeutic target or prognosis marker in both tumor and infection context.

P.B2.07.16

Tumor-associated macrophages in rectal cancer polarize to the proinflammatory M1 phenotype after irradiation in patients and 3D co-culture model

V. Stary, *D. Unterleuthner, B. Wolf, J. Strobl, A. Beer, H. Dolznig, M. Bergmann;
Medical University of Vienna, Vienna, Austria, Vienna, Austria.*

Tumor-associated macrophages initiate anti-tumoral (M1) or immunosuppressive (M2) responses depending on their polarization status. To test the effects of radiotherapy, we *ex vivo* irradiated tissue samples of human rectal cancer and assessed the phenotype of macrophages, T cells and NK cells by flow cytometry. We evaluated their distribution after short course radiotherapy (n=45) and compared findings to non-pretreated rectal cancer (n=25) using an immunostaining approach. We further investigated the influence of cancer-associated fibroblasts and cancer cells on the polarization of macrophages after *ex vivo* irradiation using 3D co-culture models. Irradiated rectal cancer samples contained less $CD68^+$ macrophages (18.2.±2 vs 12.6±3%$CD68^+$/total leukocytes) with a viability of >92% in both groups. Stainings of markers associated with the M1- (CD64, CCR7, iNos, TNFα, HLA-DR, CD86) or M2-like (CD206, CD163, IL-10, IL-4) phenotype revealed an increase of M1/M2-ratio arguing for a shift from M2- to M1-like macrophages due to irradiation. Irradiated tissue sections demonstrated diminished T cell counts (109.7±8.68 vs 45.7±17.26 $CD3^+$cells No./mm^2) but elevated infiltration of NK cells (50.3±15.51 vs 75.9±9.43 $CD56^+CD3^-$cells No./mm^2). Irradiation of 3D co-culture models led to a dose dependent increase of M1/M2. Untreated macrophages in co-cultures without fibroblasts tended to be less M2 but more M1-like. Neutralizing IL-10 antibody induced M1-like macrophages. Treatment with recombinant IL-10 partly rescued the effects of irradiation. Our findings highlight macrophages as effector cells upon irradiation by enhancing their anti-tumoral activities and diminishing their immunosuppressive behavior. This study provides a rationale for future investigation aiming for immune-modulation of macrophages to ensure optimal anti-cancer immune-activation.

P.B2.07.17

Macrophage subpopulations acquire distinct education programming along radiation treatment response in gliomas

J. Tessier, *J. Gadiot, S. Handgraaf, L. Akkari;
Netherlands cancer institute (NKI), Amsterdam, Netherlands.*

Glioblastoma (GBM) is the most malignant form of adult brain tumors. Due to resistance and tumor regrowth, overall survival only reaches 12-15 months. Interestingly, up to 30% of the tumor bulk consists of tumor-associated macrophages (TAMs). Their increase in number correlates with poor prognosis, and may therefore participate in establishing a favorable environment supporting treatment resistance and recurrence emergence. Our data support a progressive effect of radiation, a standard of care treatment for GBM, on the two subpopulations of TAMs found in GBM: microglia, the brain resident macrophages and the tumor infiltrating bone marrow-derived macrophages (BMDM). We aim to decipher how BMDM and MG are altered when in contact with GBM cell, and their respective phenotype modification after radiation. We made use of a spontaneous mouse model of GBM, faithfully recapitulating the human pathology, and performed RNA sequencing on TAMs isolated post radiation. While these two populations share similar pro-tumorigenic functions, they also differ in their response to treatment Our preliminary data show similar activation of both macrophage subpopulations, with a shift toward an M2-like phenotype in the early onsets after treatment, and acquisition of a common phenotype at recurrence. However, these phenotypes differ in intensity, indicating that despite a common education, MG and BMDM react differently to irradiation. We now aim to further understand the molecular mechanisms underlying BMDM and MG influence on GBM recurrence. These research questions will shed light on the heterotypic communication between glioma cells and their local and systemic environment.

P.B2.07.18

The inflammatory threshold guides tissue remodeling: the example of germ-free animal conventionalization and induced colitis

L. E. Vannucci[1], *F. Caja*[1,2], *D. Stakheev*[1,2], *O. Chernyavskiy*[3], *P. Tenti*[1,2], *P. Lukac*[1], *L. Rajsiglova*[1,2], *T. Hudcovic*[1], *R. Stepankova*[1], *H. Kozakova*[1], *J. Dvorak*[1], *J. Krizan*[1], *P. Sima*[1], *P. Makovicky*[4,5], *R. Sedlacek*[4,5], *P. Makovicky*[6];
[1]*Institute of Microbiology of the CAS, v.v.i., Prague 4, Czech Republic,* [2]*Faculty of Science, Charles University, Prague, Czech Republic,* [3]*Institute of Physiology of the CAS, v.v.i., Prague 4, Czech Republic,* [4]*Institute of Molecular Genetics of the CAS, v.v.i., Prague 4, Czech Republic,* [5]*Czech Centre for Phenogenomics, Vestec, Czech Republic,* [6]*Selye Janos University, Komarno, Slovakia.*

Conventional (CV) and germ-free (GF) animals offer a potent model for understanding the cross-talk between immunity and tissue structure modeling, e.g. in the colon. The constant immune activation sustained by colon microbiota can be put in comparison with immune activation of the GF animal naïf mucosal system variously induced (bacteria, dextran sodium sulfate, azoxymethane). After induction, the bowel was harvested at established time points. Samples were taken for histology and second-harmonic generation (SHG) analysis by multi-photon confocal microscopy. Mucosa samples were analyzed for cytokine expression (ELISA, PCR). The healthy CV rat revealed higher pro-inflammatory cytokine (IFNγ, IL1, TNFα) and TGFβ expression in the mucosa than the GF rat, and also different collagen stroma texture. Highly dynamic scaffold remodeling resulted in the animals induced to inflammation or bacterial colonization. The induced inflammation thickened and quickly expanded the scaffold in GF animals, without significant alterations in its general architecture. The contrary was in CV animals. The importance of different threshold of immune activation can explain the differences between the two conditions. This is an evidence of the microbiota role on colonic structure maturation through promotion of immune activation. Depending on the basal "inflammatory threshold" the scaffold cannot adapt, as seen in CV animals. Similarly, transformed cells can alter the scaffold by continuous pro-inflammatory stimulations, generating the tumor microenvironment.
Acknowledgements: institutional grant RVO 61388971; UniCredit Bank Czech Republic a.s., Italian-Czech Commercial Chamber, ITALINOX s.r.o., Manghi Czech Republic s.r.o, Paul's Bohemia s.r.o. (CZ), and MEYS LM2015040 (Czech Centre for Phenogenomics)

P.B2.07.19

Oxidative stress in the microenvironment of B cell chronic lymphocytic leukemia

M. Firczuk[1], *A. Goral*[1], *A. Muchowicz*[1], *K. Fidyt*[1], *S. Gobessi*[2], *D. Efremov*[2];
[1]*Medical University of Warsaw, Warsaw, Poland,* [2]*International Centre for Genetic Engineering & Biotechnology, Trieste, Italy.*

Modern immunotherapies are less effective in B cell chronic lymphocytic leukemia (CLL) than in other malignancies. This may be partially caused by highly immunosuppressive microenvironment observed in CLL. Several immune dysfunctions occur in CLL including impairments in T cell and myeloid cell populations. In a subset of CLL patients, elevated oxidative stress is accompanied by dysfunctions of cytotoxic T cells and may be one of the triggers of immunosuppression. However, the causes of dysregulated redox homeostasis in CLL are not well understood. We employed Eμ-TCL1 mice model of CLL to investigate the role of selected populations of immune cells in the generation of oxidative stress and immune evasion in CLL. To measure the levels of reactive oxygen species (ROS) in defined subpopulations of immune cells we used CM-H_2-DCFDA fluorescent probe and flow cytometry analysis. To assess the amounts of secreted H_2O_2 we used Amplex Red assay.
In spleens and lymph nodes of TCL1 leukemic mice we observed increased percentages of immunosuppressive populations of cells such as regulatory T cells, CD11b+/MHC-II^{low}/PD-$L1^{high}$ and, CD11c+/MHC-II^{low}/PD-$L1^{high}$. In addition, we detected elevated ROS levels in malignant B cells as well as in CD11b+ and CD11c+ cells isolated from the spleens of Eμ-TCL1 mice. Accordingly, our preliminary data suggest that Eμ-TCL1 mice-derived splenocytes produced more H_2O_2 as compared to their equivalents from non-leukemic mice. Further studies are underway to better define pathways responsible for increased ROS levels in the selected populations of immune cells.
Funding: Polish National Science Centre 2016/21/B/NZ7/02041, European Commission Horizon 2020 Programme 692180-STREAMH2020-TWINN-2015

P.B2.07.20

Characterisation of human T helper-like regulatory T cells and chemokine expression in oral squamous cell carcinoma

*M. Fraga[1], M. Yañez[2], E. Castro[1], R. McGregor[3], M. Romano[3], M. Sherman[4], M. Vidal[5], G. Cabrera[5], G. Lombardi[3], **E. Nova-Lamperti**[1];*
[1]Molecular and Translational Immunology Laboratory, Department of Clinical Biochemistry and Immunology, Universidad de Concepcion, Concepcion, Chile, [2]Unidad de Anatomía Patológica, Hospital Las Higueras, Talcahuano, Chile, [3]MRC Centre for Transplantation, School of Immunology & Microbial Sciences, King's College London, Guy's Hospital, SE1 9RT, London, United Kingdom, [4]Unidad de Anatomía Patológica, Hospital Guillermo Grantt Benavente, Concepcion, Chile, [5]Departamento de Ingeniería Informática y Ciencias de la Computación, Facultad de Ingeniería, Universidad de Concepción, Concepcion, Chile.

Regulatory T-cells (Tregs) are a subset of CD4+ T-cells that maintain immunological tolerance and regulate immune homeostasis. Tregs have been classified as regulatory T helper (Th)-like cells according to the expression of specific transcription factors, cytokines and chemokine receptors that mirror effector Th lineages. We have recently characterised peripheral blood and tissue resident Th-like Tregs in healthy donors and patients with cancer. Our results revealed that CCR4-expressing Th2-like Tregs were enriched in tumorigenic areas compared to healthy tissues. However, it is not clear whether Tregs migrate in response to specific chemokines or whether the environment supports the differentiation of Th2-type Tregs. Chemotactic assays demonstrated that Th2-like Tregs migrate preferentially to chemokines CCL17 and CCL22. However, CCR8, a chemokine receptor associated with tumour-infiltrating Tregs, was also preferentially expressed in Th2-like Tregs, therefore it is also possible that Th2-like Tregs migrate in response to CCL1 and CCL18. In this study, we evaluated the expression of CCL1, CCL17, CCL18 and CCL22 in cancer tissues from patients with oral squamous cell carcinoma compared to healthy oral mucosa. Our results demonstrated that patients with oral cancer expressed higher levels of CCL18 and CCL17. These results suggest that both, CCR4 and CCR8, can support the migration of Th2-like Tregs to malignant areas in oral cancer.

P.B3.01 T-cell regulation - Part 1

P.B3.01.01

Abundance of Tregs and effects of their inhibition in oral cancer

***S. Aggarwal**, S. Sharma, S. Das;*
All India Institute of Medical Sciences, Delhi, India.

Oral squamous cell carcinoma (OSCC) is one of the major cancers affecting in Asian countries. The main causative factor has been tobacco habit. It has been reported that immune dysfunction in these patients is one of the major factors for tumor growth and dissemination that affects disease-free survival of the patients. We assessed the phenotypic and functional characteristics of Regulatory T (T_{reg}) $CD4^+CD25^+FoxP3^+$subsets in patients with OSCC by multicoloured flow cytometry. The effects of garcinol mediated, MACS-purified Tregs inhibition on growth of cell lines was also studied. An increased frequency of $CD4^+CD25^+$, $CD4^+FoxP3^+$, $CD8^+FoxP3^+$ and $CD4^+CD25^+FoxP3^+$ was observed in the peripheral circulation of OSCC patients that positively correlated with clinicopathological features. The increased frequency of $CD4^+CD8^+CD25^+FoxP3^+$, CTLA-4^+, $GITR^+$, $NrP1^+$, HLA-DR^+, $CD127^+$, $Tbet^+$ and granzyme B^+ (GzmB) T_{regs} alsoshowed a significantly higher prevalence in OSCC patients. Functionally $CD4^+FoxP3^+T_{regs}$ showed skewed expression of IL-2, IL-10 and IL-35 in patients as compared with the normal controls. Further, enhanced expression of CCR5 and CCR7 on T_{regs} with up regulation of their ligands (CCL5, CCL19 and CCL21) in tumor cells indicates efficient recruitment and trafficking of T_{regs} to the tumor site. Additionally, garcinol treatment significantly ($P < 0.001$) inhibited the growth of OSCC cells with a concomitant induction of apoptosis, cell cycle arrest and anti-angiogenesis. It appears that garcinol concurrently prevents many tumour-promoting effects Treg. Hence, modulation of functional dynamics of selective T_{reg} subsets may be useful in enhancing anti tumor immunity and developing immunotherapeutic strategies for patients with oral squamous cell carcinoma.

P.B3.01.02

CD39+ regulatory T cells accumulate in colon adenocarcinomas and display markers of increased suppressive function

***F. Ahlmanner**[1], P. Sundström[1], P. Akeus[1], J. Eklöf[1], L. Börjesson[2], B. Gustavsson[2], E. Bexe Lindskog[2], S. Raghavan[1], M. Quiding-Järbrink[1];*
[1]Dept of Microbiology and Immunology, Gothenburg, Sweden, [2]Dept of Surgery, Gothenburg, Sweden.

Increasing knowledge of the function and regulation of tumor-infiltrating lymphocytes has led to new insights in cancer immunotherapy. Regulatory T cells (Treg) accumulate in colon tumors, and we recently showed that $CD39^+$ Treg from cancer patients inhibit transendothelial migration of conventional T cells. CD39 mediates the hydrolysis of ATP to immunosuppressive adenosine and adds to the immunosuppressive effects of Treg. Here, we further investigated the regulatory features of intratumoral $CD39^+$ Treg in colon cancer. Using flow cytometry analyses of cells from 46 colon cancer patients, we confirmed the accumulation of $CD39^+$ Treg in the tumor tissue compared to unaffected colon tissue, and also observed a positive correlation between CD39 expression among intratumoral Treg and CD39 expression among circulating Treg ($p < 0.01$). Furthermore, tumor-infiltrating Treg express significantly more CD39 and Foxp3 on a per cell basis, as well as markers indicating increased turnover and suppressive function, such as Ki67, ICOS, PD-L1 and CTLA-4. Functional suppression assays suggest potent suppressive capacity of $CD39^+$ Treg on proliferation and IFN-γ secretion by conventional T cells. Preliminary studies also indicate that high levels of CD39 expression among intratumoral Treg may correlate to a worse patient outcome. In conclusion, our results show a large infiltration of $CD39^+$ Treg in colon tumors, and this subset appear more immunosuppressive than their $CD39^-$ counterparts. We suggest that immunotherapy aimed at reducing tumor-infiltrating $CD39^+$ Treg activity may be particularly useful in the setting of colon cancer.
Funding sources: Swedish Research Council and Swedish Cancer Foundation.

P.B3.01.03

Lymphopenia induces homeostatic T-cell proliferation after autologous stem cell transplantation

***M. Baliu-Piqué**[1], V. van Hoeven[2], J. Drylewicz[1], L. E. van der Wagen[1], A. Janssen[1], L. J. Ackermans[1], S. A. Otto[1], J. H. Kuball[1], J. A. Borghans[1], K. N. Tesselaar[1];*
[1]University Medical Center Utrecht, Utrecht, Netherlands, [2]Academic Medical Center, Amsterdam, Netherlands.

Hematopoietic stem cell transplantation (SCT), an increasingly common treatment for many types of cancer and immune disorders, comes at a cost of lymphopenia and the concomitant need for immune reconstitution. A successful reconstitution depends on the early recovery of T-lymphocytes. However, T-cell recovery after SCT generally occurs extremely slowly. There is evidence in mice that lymphocyte production and/or survival rates increase when cell numbers are low. Studies in humans have suggested that increased T-cell proliferation after SCT is associated with clinical events (GVHD) rather than with low lymphocyte numbers. Here, we investigated whether T-cell production is also increased under lymphopenic conditions in humans. Using *in vivo* deuterium labelling and mathematical modelling we quantified the dynamics of T-cells in patients who underwent autologous SCT, and had no signs of infectious complications. After a reconstitution period of up to 1.5 years, absolute numbers of $CD4^+$ T-cells, particularly of the naive subset, were still very low in these patients. Deuterium labelling demonstrated that the production rates of naive and memory $CD4^+$ and $CD8^+$ T-cells in SCT patients were significantly increased compared to healthy individuals. TREC content analysis and CD31 expression of naive $CD4^+$ T-cells suggested that these increases in T-cell production were due to increased peripheral proliferation, and not thymic output. Taken together, this work shows that despite the slow reconstitution of lymphocyte numbers after SCT in humans, lymphocyte proliferation rates are in fact homeostatically increased in response to lymphopenia, bringing new insights in the cellular dynamics behind a timely T-cell reconstitution.

P.B3.01.04

Tyrosine kinase inhibitor Dasatinib effects on iNKT cells and innate CD8 T-cells in chronic myeloid leukemia patients

***A. Barbarin**[1], L. Lefevre[1], M. Abdallah[1], N. Piccirilli[1,2], E. Cayssials[1,2], L. Roy[3], A. Herbelin[1], J. M. Gombert[1,2,4];*
[1]INSERM U1082, Poitiers, France, [2]CHU de Poitiers, Poitiers, France, [3]Hopital Henri Mondor, Créteil, France, [4]Université de Poitiers, Poitiers, France.

Introduction: Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell malignancy caused by the presence of the chimerical BCR-ABL oncoprotein, with deregulated tyrosine kinase (TK) activity. Dasatinib is a highly potent second-generation BCR-ABL tyrosine kinase inhibitor (TKI) with off-target immunological effects. We recently identified in healthy individuals a distinct new innate CD8 T-cell subset characterized by the expression of NK receptors (KIR/NKG2A), high Eomesodermin expression and prompt IFN-γ production in response to innate-like stimulation by IL-12+IL-18. Like iNKT cells, innate CD8 T-cells are severely reduced and functionally deficient in CML patients at diagnosis and partially restored after therapy with Imatinib, a first-generation TKI. Here, we tested the hypothesis of a direct effect of Dasatinib on iNKT cells and innate CD8 T-cells.
Methods: Using flow cytometry, we analyzed iNKT cells and innate CD8 T-cells in the peripheral blood from patients treated 12 months with Dasatinib. In a mouse model, we tested Dasatinib effects both *in vivo* (oral gavage) and *in vitro* using thymocyte and splenocyte culture systems.
Results: In CML patients treated 12 months with Dasatinib, we observed an increased percentage of both iNKT cells and innate CD8 T-cells. In the mouse model, *in vivo* and *in vitro* analysis showed an increase of iNKT cells with a Th1 profile. Despite the decrease of the memory CD8 T-cell compartment, the innate-memory CD8 T-cell pool was increased.
Conclusion: Taken together, our data are in favor of a direct effect of Dasatinib on both iNKT cells and innate CD8 T-cell numbers and functions.

P.B3.01.05

Unconventional T-αβ cells (iNKT and innate CD8 T cells) in solid tumors and their tumor environment

A. Barbarin[1], *B. Morin*[1], *N. Piccirilli*[1,2], *N. Gonzalo Nunez*[3], *E. Piaggio*[3], *V. Lavoue*[4], *V. Catros*[4], *A. Herbelin*[1], *J. M. Gombert*[1,2,5];
[1]*INSERM U1082, Poitiers, France,* [2]*CHU de Poitiers, Poitiers, France,* [3]*CICBT 1428 SiRIC, institut Curie, Paris, France,* [4]*CHU de Rennes, INSERM U991, CRN de Rennes, Rennes, France,* [5]*Université de Poitiers, Poitiers, France.*

Introduction: Cancer immuno-surveillance involves innate and adaptive cells as well as non-conventional T cells like iNKT cells. Recently, we have identified in humans a new CD8 T-cell subset, named innate CD8 T-cells, expressing a classical TCR-αβ and NK markers and responding to innate-like stimulation by the pro-inflammatory cytokines IL-12, IL-18 and IL-33. Our recent data support a possible link between iNKT cells and innate CD8 T-cells *via* the secretion of IL-4 by iNKT cells. Here, our aim was to study the iNKT/innate CD8 T-cell axis in solid tumors.
Methods: We analyzed by flow cytometry blood samples, ascites, pleural fluid, carcinosis and tumor samples from patients with ovarian, breast, pancreas or colon cancer for the presence and activation state of iNKT cells and innate CD8 T-cells. IL-12, IL-18 and IL-33 levels were measured in plasma and ascite supernatants by ELISA.
Results: iNKT cells and Innate CD8 T-cells were present in the tumor, carcinosis and ascites. iNKT cells from the tumor and tumor environment were enriched in CD69 positive cells, reflecting their higher activation state than their peripheral counterparts. Furthermore, we observed a positive correlation between the expression of the two transcriptional factors characterizing iNKT and innate CD8 T-cells, PLZF and Eomes, respectively. Finally, we showed that the tumor environment was enriched in IL-12 and IL-33 whereas IL-18 was detected at the periphery.
Conclusion: Taken together, our results support the hypothesis of anti-tumoral cooperation between iNKT cells and innate CD8 T-cells in response to the cytokines IL-12 and IL-33 in solid tumors.

P.B3.01.06

Regulatory T-cell compartment in HIV-1+ pregnancies loses gestation adaptations and function

A. Cocker[1], *S. Sivarajasingam*[1], *A. Sassine*[1], *I. Raj*[2], *S. Dermont*[2], *A. Khan*[2], *N. Imami*[1], *M. Johnson*[1];
[1]*Imperial College London, London, United Kingdom,* [2]*Chelsea and Westminster Hospital, London, United Kingdom.*

Regulatory T cells (Treg) are thought to maintain tolerance towards the fetus during pregnancy, and mediate responses to viruses such as Cytomegalovirus (CMV) that risk disrupting immunological balance. HIV-1+ women have higher incidences of preterm labour, potentially caused by increased immune activation. Here the longitudinal development of the Treg compartment and its relation to viral responses in HIV-1- and HIV-1+ pregnancies is compared.
Peripheral blood mononuclear cells were isolated from pregnant HIV-1+ (n=19) and HIV-1- (n=15) women, and flow cytometric analysis performed to determine the frequency of Treg cells (CD3+CD4+CD25+CD127^{low}) and their expression of CD45RA, and HLA-DR. IFNγ and IL-2 ELISpot assays were completed to quantify CMV specific responses. Statistical analysis was carried out using Prism version 7.0. Spearman's rank correlation and linear regression were applied, and statistical significance defined as p≤0.05. Point estimates for gestational changes were derived using MIXED procedure in SAS version 9.4.
Treg frequency of CD4+ T cells and CD45RA expressing cells increased with gestation in HIV-1- women. Treg expressing HLA-DR decreased with gestation. None of these changes were seen in the HIV-1+ group. IFNγ and IL-2 responses to CMV were negatively correlated with Treg frequency and CD45RA, and positively with HLA-DR expression in HIV-1- participants. Only HLA-DR and IFNγ demonstrated a positive relationship in the HIV-1+ women.
The Treg compartment demonstrates gestational developments and correlates to anti-viral responses, supporting their suppressive function. Loss of these relationships in HIV-1+ participants highlights this compartment as clinically relevant to their increased preterm labour rate.

P.B3.01.07

Role of the interleukin-33/ ST2L-axis for the CD8-dependent anti-cancer cytotoxicity

C. Dreis[1], *F. Ottenlinger*[1], *M. Herrero San Juan*[1], *M. Putyrski*[2], *A. Ernst*[2], *M. U. Martin*[3], *J. M. Pfeilschifter*[1], *H. H. Radeke*[1];
[1]*pharmazentrum frankfurt, Frankfurt am Main, Germany,* [2]*Institute of Biochemistry II, Frankfurt am Main, Germany,* [3]*Immunology FB08 Justus-Liebig-University, Giessen, Germany.*

Novel cancer therapies target the activation of tumor-antigen specific cytotoxic T cells to improve treatment efficacy. For the alarmin Interleukin-(IL-)33, ligand of Th2 marker ST2L and IL-1 family member, increasing evidence suggests an involvement in Th1 immunity. We previously demonstrated IL-33/ IL-12-dependent co-activation of murine cytotoxic T cells and hypothesize induction of anti-tumoral Th1 activity by IL-33. We re-evaluated the regulation of IL-33 bioactivity within optimized *in vitro* bioactivity assays and analyzed regulation of ST2L/ sST2 expression of human immune cells.
IL-33 aa (amino acid) 111-270 and recombinantly generated hyperactive aa95-270 potently activated ST2L-expressing HEK293 reporter cells. In a competitive assay with ST2L, significant downregulation of IL-33 bioactivity required 100-fold excess of soluble decoy receptor sST2. However, in the absence of ST2L, aa111-270 and aa95-270 exhibited high binding affinities to sST2 (2.21 nM ± 1.1; 13.47 ± 2.9 nM). IL-33 and IL-1β detected by ELISA in serum of healthy donors (n=30) failed to induce a corresponding bioactivity. Still, bioactivity of exogenous recombinant IL-33, but not IL-1β, was significantly reduced in human plasma. Proteases and oxidation have been excluded as elicitors of this effect.
Peripheral blood mononuclear cells and isolated CD8+ T cells revealed expression of cell surface ST2L as well as sST2 mRNA. These results support our hypothesis of a crucial role of IL-33 in the activation of Th1 immunity, followed by a systemic, strongly regulated inactivation. Understanding the regulation of IL-33 bioactivity will lead to optimized activation of tumor-antigen specific activation of cytotoxic T cells. Funded by Else-Kröner-Fresenius Stiftung.

P.B3.01.08

The role of the PD-1 inducible ARF-like GTPase 4d in immune inhibition of anti-tumor CD8 T cell immunity

B. Geers*, P. Sprezyna, J. Endig, L. Diehl;*
University Medical Center Hamburg-Eppendorf, Institute of Experimental Immunology and Hepatology, Hamburg, Germany.

Blockade of the inhibitory PD-L1/PD-1 pathway holds promise for cancer immunotherapy. Priming of CD8 T cells by liver sinusoidal endothelial cells (LSEC) leads to development of T cells with memory function but lacking immediate effector function, which depended on PD-L1 signaling and restriction of IL-2 availability. We previously found that the small GTPase Arl4d was PD-L1 dependently highly induced in naïve CD8 T cells and that Arl4d functions to repress their production of IL-2. As PD-L1/PD-1 signaling may inhibit effector function of anti-tumor CD8 T cells, we aimed to investigate whether Arl4d is involved in PD-1 mediated inhibition of anti-tumor CD8 T cell responses *in vivo*. Therefore, we analyzed B16-OVA melanoma tumor growth after s.c. injection into male *Arl4d*$^{+/+}$ or *Arl4d*$^{-/-}$ mice. All mice developed solid tumors within ~14d, but Arl4d-deficiency led to a significant decrease in tumor size and weight. We, furthermore, found high percentages of tumor infiltrating CD44^{+}CD62L^{-}CD8^{+} T cells expressing PD-1 in both *Arl4d*$^{+/+}$ or *Arl4d*$^{-/-}$ mice. We did not, however, observe differences in CD8 T cell numbers or activation state in the tumor nor in the draining lymph nodes. We demonstrate that Arl4d-deficiency leads to a better control of B16-OVA tumor growth in mice but did not find any overt changes in the intra-tumoral and tumor-draining T cell compartment. To identify the mechanism responsible for the decreased tumor growth in *Arl4d*$^{-/-}$ mice, a more detailed functional and phenotypical analysis of tumor infiltrating CD8 T cells but also other immune cells in the tumor may be needed.

P.B3.01.09

Evaluating *in vivo*anti-tumor T cell responses to melanoma using newly developed tumor cell lines

J. L. Hope*, M. L. Barraza, R. Tinoco, L. M. Bradley;*
Sanford Burnham Prebys Medical Discovery Institute, La Jolla, United States.

Mouse models of cancer remain the preferred means to assess the efficacy of anti-cancer therapies, and several models have been used to address the effect of cancer drugs or in the discovery of biomarkers. The YUMM1.5 and YUMMER1.7 murine tumor models were developed from the *Braf/Pten* engineered mouse model to more closely reflect melanomas that are driven by human disease-relevant mutations; however, the dominant T cell-recognized epitopes specific for these tumors remains unknown. We therefore sought to engineer OVA-expressing versions of the YUMM1.5 and YUMMER1.7 tumor lines, hereafter referred to as YUMM1.5-OVA and YUMMER1.7-OVA. Using stable transfection to insert a plasmid expressing full-length secretory ovalbumin, we have generated polyclonal and monoclonal cell lines that generate an *in vivo* OVA-specific response as validated by MHC class I tetramer staining and cytokine production following OVA peptide stimulation. Together, these two new models for melanoma will allow us to assess intrinsic and extrinsic mechanisms regulating the generation of effective and recovery of exhausted T cell responses to melanoma *in vivo*.

P.B3.01.10

Immunogenicity and immunophenotypes in young-onset colorectal cancers

M. E. Ijsselsteijn*, T. Brouwer, D. Ruano, R. van der Breggen, H. Morreau, K. Jordanova, N. De Miranda;*
Leiden university medical centre, Leiden, Netherlands.

Immunotherapy has emerged as one of the most promising options for cancer treatment. Tumour-infiltrating immune cells have great prognostic value in solid tumours, including colorectal cancer. In recent years, a tendency has been observed towards an increased incidence of colorectal cancer in young (<50) patients. These patients are not included in screening programs and thus often diagnosed at advanced stages of tumour progression. To our knowledge, no study has yet characterized immunophenotypes and immune evasive mechanisms in young-onset colorectal cancers. To that end, we investigated the expression of HLA class I and PD-L1 by Immunohistochemistry in over 200 young-onset colorectal cancers. Furthermore, we applied a novel multispectral immunofluorescence technique to simultaneously assess 7 T-cell related markers.

We describe that HLA class I is maintained in 62% of mismatch-repair proficient tumours, which allows the development of neo-antigen targeted therapies, but lost in 78% of mismatch-repair deficient tumours. Interestingly, reduced, but not lost, HLA class I expression was specifically observed in liver metastasis, which suggests a specific pressure that could be utilised for immunotherapeutic exploitation. As previously demonstrated, PD-L1 expression is limited and often restricted to immune cell compartments. Multispectral immunofluorescence imaging allows for a comprehensive overview of T-cell immunophenotypes and their relation to HLA class I expression in young-onset colorectal cancer. Retained HLA class I expression in the majority of colorectal cancers associated with low infiltration by effector immune cells suggests the possibility for therapeutic induction of anti-tumour immune responses in young-onset colorectal cancers, for instance, by means of neo-antigen-targeted therapies.

P.B3.01.11

GITR targeting enhances functionality of tumor-infiltrating T cells in hepatocellular carcinoma

*A. A. van Beek[1], G. Zhou[1], M. Doukas[1], P. P. Boor[1], L. Noordam[1], S. Mancham[1], L. Campos Carrascosa[1], M. van der Heiden-Mulder[1], W. G. Polak[1], J. N. IJzermans[1], Q. Pan[1], C. Heirman[2], A. Mahne[3], S. L. Bucktrout[3], M. J. Bruno[1], D. Sprengers[1], **J. Kwekkeboom**[1];*
[1]Erasmus MC - University Medical Centre, Rotterdam, Netherlands, [2]Vrije Universiteit, Brussels, Belgium, [3]Rinat Laboratories, Pfizer Inc., South San Francisco, United States.

No curative treatment options are available for advanced hepatocellular carcinoma (HCC). A recent study demonstrated that anti-PD1 antibody therapy can induce tumor regression in 20% of advanced HCC patients, thereby revealing that co-inhibitory immune checkpoint blockade may have therapeutic potential for this type of cancer. However, whether agonistic targeting of costimulatory receptors can stimulate anti-tumor immunity in HCC is as yet unknown. We studied expression of the co-stimulatory receptor GITR on tumor-infiltrating lymphocytes (TIL) isolated from freshly resected tumors, and on lymphocytes isolated from paired tumor-free liver tissues and blood of HCC patients. We determined whether agonistic targeting of GITR could enhance ex vivo functional responses of HCC TIL. In all three tissues, GITR was predominantly expressed on $CD4^+FoxP3^+$ regulatory T cells (Treg). The highest expression levels were found on $CD4^+FoxP3^{hi}CD45RA^-$ activated Treg in tumors. Addition of recombinant GITR-ligand or a humanized agonistic antibody against GITR (10H2#13, Pfizer) to *ex vivo* cultures of HCC TIL enhanced $CD8^+$ T cell proliferation, granzyme B and IFN-γ production, in response to CD3/CD28 stimulation. GITR ligation also enhanced proliferative responses of tumor-derived $CD4^+$ and $CD8^+$ TIL to tumor antigens presented by mRNA-transfected autologous B cell blasts. Interestingly, GITR-expressing Treg, CD4+Foxp3- T helper cells, and CD8+ T cells in tumors co-expressed PD-1. Combining GITR ligation with anti-PD1 antibody further enhanced proliferative responses of $CD4^+$ and $CD8^+$ TIL to tumor antigens compared to either single treatment. Conclusion: Agonistic targeting of GITR may be a promising strategy for single or combinatorial immunotherapy in HCC.

P.B3.01.12

Thymus derived Treg cell development is regulated by C type lectin mediated BIC miRNA155 expression

*R. Sánchez Díaz[1], R. Blanco Dominguez[1], S. Lasarte[1], K. Tsilingiri[1], E. Martín Gayo[2], **B. Linillos Pradillo**[1], H. de la Fuente[3], F. Sánchez Madrid[3], R. Nakagawa[4], M. Toribio[5], P. Martin[1];*
[1]CNIC, Madrid, Spain, [2]Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas, Universidad Autónoma de Madrid, Madrid, Spain, [3]Hospital de la Princesa, Madrid, Spain, [4]The Francis Crick Institute,, London, United Kingdom, [5]CIBER de Enfermedades Cardiovasculares, Instituto de Salud Carlos III, Madrid, Spain.

BACKGROUND AND OBJECTIVE: Thymus-derived regulatory T (tTreg) cells are key to preventing autoimmune diseases, but the mechanisms involved in their development remain unsolved. Here, we show that the C-type lectin receptor CD69 controls tTreg cell development and peripheral Treg cell homeostasis through the regulation of BIC/microRNA 155 (miR-155) and its target, suppressor of cytokine signaling 1 (SOCS-1).
METHODS: Using Foxp3-mRFP/*cd69*$^{+/-}$ or Foxp3-mRFP/*cd69*$^{-/-}$ reporter mice and short hairpin RNA (shRNA)-mediated silencing and miR-155 transfection approaches, we found that CD69 deficiency impaired the signal transducer and activator of transcription 5 (STAT5) pathway in $Foxp3^+$ cells
RESULTS: This results in BIC/miR-155 inhibition, increased SOCS-1 expression, and severely impaired tTreg cell development in embryos, adults, and Rag2$^{-/-}$ γc$^{-/-}$ hematopoietic chimeras reconstituted with *cd69*$^{-/-}$ stem cells. Accordingly, *mirn155*$^{-/-}$ mice have an impaired development of $CD69^+$ tTreg cells and overexpression of the miR-155-induced CD69 pathway, suggesting that both molecules might be concomitantly activated in a positive-feedback loop. Moreover, *in vitro*-inducible $CD25^+$ Treg (iTreg) cell development is inhibited in *Il2rγ*$^{-/-}$/*cd69*$^{-/-}$ mice.
CONCLUSIONS: Our data highlight the contribution of CD69 as a nonredundant key regulator of BIC/miR-155-dependent Treg cell development and homeostasis.

P.B3.01.13

GMP-compliant generation of human granzyme B^+ regulatory B cells for the therapy of graft-versus-host disease

***C. Mangold**[1], A. Felsen[1], L. Kurz[1], T. Trzaska[1], P. Reinhardt[1,2], H. Schrezenmeier[1,2], D. Fabricius[3], K. Schilbach[4], B. Jahrsdörfer[1,2];*
[1]Department of Transfusion Medicine, Ulm, Germany, [2]Institute for Clinical Transfusion Medicine and Immunogenetics, German Red Cross Blood Transfusion Service Baden-Württemberg – Hessen and University Hospital Ulm, Ulm, Germany, [3]Department of Pediatrics, Ulm, Germany, [4]Department of General Pediatrics, Oncology/Hematology, Eberhard-Karls University Tübingen, Tübingen, Germany.

Granzyme B (GrB)-secreting regulatory B cells (B_{reg}) suppress T cell proliferation by GrB-mediated degradation of the T cell receptor and are involved in various pathologies. Their exploitation as novel cell-therapeutic agents may therefore represent a promising approach for the treatment of *graft-versus-host disease* (GvHD*)*. Recently, we developed a cocktail consisting of IL-21 and antibodies against the human B cell receptor, allowing for easy *ex-vivo* induction of GrB^+ B_{reg} from peripheral B cells isolated from whole blood. In our current study, we now used a GMP-compliant positive selection kit to directly isolate $CD19^+$ B cells from leukapheresis products collected from eight unstimulated healthy donors. Subsequently, we tested the isolated B cells in terms of their potential to differentiate into B_{reg}. On average, we were able to isolate $56.5x10^6$ B cells from ~$6.6x10^8$ total PBMC. Purity was 99.4%, viability was 98.1%. Extrapolated to the size of a full leukapheresis product, the generation of >$600x10^6$ B_{reg} is possible. After 48 hours of stimulation, an average of 64.7% of B cells showed the typical GrB^+ phenotype. Of note, these B cells maintained their B_{reg} phenotype for up to another 72 hours after the end of stimulation. In conclusion, our findings demonstrate that GMP-compliant generation of induced B_{reg} is feasible. Our results pave the way for further development of B_{reg} as novel cell-therapeutic agent. A first pilot study on the effect of GrB^+ B_{reg} on GvHD in a humanized mouse model will be starting in June 2018. Initial results will be discussed on the conference.

P.B3.01.14

MicroRNA-31 is induced in repeatedly activated T Helper 1 Lymphocytes and regulates their motility

*M. Bardua[1], C. Haftmann[1], P. Durek[1], M. Mcgrath[1], C. Tran[1], G. Heinz[1], P. Maschmeyer[1], M. Lohoff[2], H. Chang[1], A. Radbruch[1], **M. Mashreghi**[1];*
[1]Deutsches Rheuma-Forschungszentrum, Berlin, Germany, [2]Institute for Medical Microbiology and Hospital Hygiene, University of Marburg,, Marburg, Germany.

T helper (Th) lymphocytes can be readily found in inflamed tissues of patients with rheumatic diseases despite immunosuppressive therapies and express the transcription factor Twist1, which is a functional biomarker for Th1 cells with a history of repeated (auto-)antigenic stimulation. Little is known about the molecular adaptations which allow these cells to persist in the inflamed tissues. To mimic Th cells from either protective immune reactions or chronic inflammation, we have generated acutely (once) and repeatedly (four times) activated Th cells *in vitro*. By performing high throughput sequencing of small RNAs, we have identified the microRNA-31 (miR-31) being selectively upregulated in repeatedly activated murine Th1 (Th1 rep.) and in memory Th lymphocytes isolated from the synovial fluid of patients suffering from rheumatic joint diseases as compared to once activated Th1 cells or memory Th cells isolated from the blood, respectively.
In order to identify direct targets and the molecular function of miR-31, we determined the transcriptomes of Th1 rep. cells after antagomir mediated miR-31 knockdown. We observed a significant enrichment of miR-31 target genes and identified the gene set “Regulation of actin cytoskeleton” to be enriched in Th1 rep cells lacking miR-31 expression. In an *in vitro* transwell migration assay Th1 rep. cells showed 50% less migration as compared to once activated Th1 cells. This reduction in the migratory capacity is partly repealed by miR31 knockdown in Th1 rep. cells. MiR-31 might represent a molecular switch important for the persistence of proinflammatory Th cells in inflamed tissues.

P.B3.01.15

Characterising immune dysregulation in the inflammatory skin disease Hidradenitis suppurativa

***B. Moran**[1], J. Musilova[2], R. Hughes[2], A. Malara[2], K. H. Mills[1], D. C. Winter[2], A. Tobin[3], B. Kirby[2], J. M. Fletcher[1];*
[1]Trinity College Dublin, Dublin, Ireland, [2]St Vincent’s University Hospital, Dublin, Ireland, [3]Tallaght Hospital, Dublin, Ireland.

Introduction: Hidradenitis suppurativa (HS) is a chronic, debilitating skin disease with 1-4% prevalence and high morbidity. Symptoms include painful lesions, which leak a bloody, suppurative, foul-smelling discharge. These lesions can merge to form dermal tunnels, leading to restricted and painful movement, and a significant reduction in quality of life. Risk factors include obesity and smoking, with a 3:1 female:male ratio. HS pathogenesis is poorly understood, with immune dysregulation strongly implicated. Materials and Methods: Cells isolated from skin biopsies and blood from HS patients and healthy volunteers were dissociated and analysed by flow cytometry to characterise infiltrating cells, and reveal their function. A fraction of biopsy was reserved for immunohistochemistry analysis. Results: We observed substantial immune cell infiltration in HS lesions, including significantly increased numbers of neutrophils, macrophages, B cells and T cells, particularly $CD4^+$ T cells. Whilst phenotypic and molecular characterisation is ongoing, we have observed a striking T_h17-skewed profile in HS skin, even in non-lesional biopsies. Regulatory T (T_{reg}) cells were also enriched; however the T_h17:T_{reg} cell ratio was highly dysregulated in favour of T_h17 cells. In contrast, lesions from anti-TNF treated HS patients exhibited a significant reduction in T_h17 cell frequency, and normalisation of the T_h17:T_{reg} cell ratio. Further, these infiltrating cells appear to have a much less inflammatory phenotype, similar to that of healthy individuals. Conclusion: These data suggest that IL-17 inhibition via TNF blockade is associated with correction of immune dysregulation in HS, and provides a rationale for targeting the IL-17 pathway in the disease.

P.B3.01.16

Regulation of Foxp3 level and suppressive activity of thymic regulatory T cells by CML-derived extracellular vesicles

J. Swatler[1], *W. Dudka-Ruszkowska*[1], *L. Bugajski*[1], *E. Kozlowska*[1,2], *K. Piwocka*[1];
[1]*Laboratory of Cytometry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland,* [2]*Department of Immunology, Faculty of Biology, University of Warsaw, Warsaw, Poland.*

Regulatory T cells constitute a significant immunosuppressive factor in BCR-ABL-positive chronic myeloid leukemia (CML), as they participate in inhibition of effector immune response against leukemic cells. As CML cells are autologous and upregulate various self-antigens, thymic Treg (tTreg), as auto-tolerant cells, are of significant interest. Immune cells can be regulated by extracellular vesicles (EVs) secreted by cancer cells, as demonstrated in solid tumors. Leukemic EVs have been shown to influence stromal and endothelial cells in bone marrow niche, but their immunomodulatory role has not been explored.
We have investigated role of leukemic EVs in differentiation and function of tTreg, using different mouse models, including *ex vivo* model of tTreg differentiation and *in vitro* suppression assay, followed by multicolor flow cytometry. EVs from 32D BCR-ABL+ cells were isolated by differential ultracentrifugation and characterized by TEM, nanoparticle tracking and western blotting.
Mature, sorted tTreg exposed to CML-derived EVs exhibit higher suppressive activity. They also express significantly higher level of Foxp3 transcription factor, which suggests global regulation of tTreg function. This effect is attenuated by BCR-ABL inhibitor (imatinib) treatment. We also show that even though CML-derived EVs do not increase tTreg differentiation *ex vivo*, naïve thymocytes exposed to these EVs during *ex vivo* culture and eventually differentiated into Tregs demonstrate higher level of Foxp3 and higher suppressive activity.
Collectively, our results demonstrate that leukemic extracellular vesicles upregulate suppressive activity of both differentiating and mature tTreg, thus suggesting a novel immunosuppressive mechanism in chronic myeloid leukemia.
Supported by National Science Center grant 2013/10/E/NZ3/00673 (KP)

P.B3.01.17

Regulatory T cells in ovarian cancer are characterized by a highly activated phenotype that is distinct from melanoma

A. Toker[1], *L. T. Nguyen*[1], *S. C. Stone*[1], *C. S. Yang*[2], *S. R. Katz*[1], *P. A. Shaw*[1], *B. A. Clarke*[1], *D. Ghazarian*[1], *A. Al-Habeeb*[1], *A. Easson*[1], *W. L. Leong*[1], *D. R. McCready*[1], *M. Reedijk*[1], *C. J. Guidos*[3], *T. J. Pugh*[2], *M. Q. Bernardini*[1], *P. S. Ohashi*[1];
[1]*University Health Network, Toronto, Canada,* [2]*University of Toronto, Toronto, Canada,* [3]*Hospital for Sick Children Research Institute, Toronto, Canada.*

Regulatory T (Treg) cells expressing the transcription factor FOXP3 are essential for the maintenance of immunological self-tolerance but play a detrimental role in most cancers due to their ability to suppress antitumor immunity. The phenotype of human circulating Treg cells has been extensively studied, but less is known about tumor-infiltrating Treg cells. We studied the phenotype and function of tumor-infiltrating Treg cells in ovarian cancer and melanoma to reveal potential Treg cell-associated molecules that can be targeted by tumor immunotherapies. Treg cells isolated from ovarian tumors displayed a distinct cell surface phenotype with increased expression of a number of receptors associated with TCR engagement, including PD1, 4-1BB and ICOS. Higher PD-1 and 4-1BB expression was associated with increased responsiveness to further TCR stimulation and increased suppressive capacity, respectively. Transcriptomic and mass cytometry analyses revealed the presence of Treg cell subpopulations and further supported a highly activated state specifically in ovarian tumors. In comparison, Treg cells infiltrating melanomas displayed lower FOXP3, PD-1, 4-1BB and ICOS expression and were not as potent suppressors of CD8 T cell proliferation. The highly activated phenotype of ovarian tumor-infiltrating Treg cells may be a key factor in the establishment of an immunosuppressive tumor microenvironment and constitute a roadblock to successful immunotherapy. Receptors that are specifically expressed by tumor-infiltrating regulatory T cells could be exploited for the design of novel combination tumor immunotherapies.

P.B3.02 T-cell regulation - Part 2

P.B3.02.01

MDSC specifically suppress IFN-γ production and antitumor cytotoxic activity of Vδ2 T-cells

A. Sacchi, N. Tumino, A. Sabatini, E. Cimini, R. Casetti, V. Bordoni, G. Grassi, ***C. Agrati****;*
National Institute for Infectious Diseases Lazzaro Spallanzani, Rome, Italy.

γδ T cells represent less than 5% of circulating T cells, they exert a potent cytotoxic function against tumor or infected cells, and secrete cytokines like conventional αβ T cells. As αβ T-cells γδ T cells reside in the typical T cell compartments (the lymph nodes, and spleen), but are more widely distributed in tissues throughout the body. For these reasons, some investigators are exploring the possibility of immunotherapies aimed to expand and activate Vδ2 T cells, or using them as CAR carriers. However, the role of immunosuppressive microenvironment on Vδ2 T cells during infections and cancers has not been completely elucidated. In particular, the effects of myeloid derived suppressor cells (MDSC), largely expanded in such pathologies, was not explored. In the present work we demonstrated that MDSC may inhibit IFN-γ production and degranulation of phosphoantigen-activated Vδ2 T cells. Moreover, the Vδ2 T cells cytotoxic activity against the burkitt lymphoma cell line Daudi and Jurkat cell line were impaired by MDSC. This impairment was not mediated by the classical MDSC inhibition pathways iNOS, IDO and ArgI. However, ArgI specific inhibition in the absence of MDSC augment Vδ2 T cell cytotoxicity, suggesting that tumor cell itself is able to partially impair Vδ2 T cell functions. These data open a key issue in the context of Vδ2-targeted immunoteraphy, suggesting the need of combined strategies aimed to boost Vδ2 T cells circumventing tumor- and MDSC-induced Vδ2 T cells suppression.

P.B3.02.02

Autosomal dominant immune dysregulation syndrome in a patient with CTLA4 deficiency

G. Aksu*, A. Aykut, E. Severcan, N. Edeer Karaca, S. Eren Akarcan, E. Parıltay, A. Durmaz, O. Cogulu, N. Kutukculer;*
Ege University School of Medicine, Izmir, Turkey.

Cytotoxic T lymphocyte antigen-4 (CTLA4) protein, is an essential negative regulator of immune responses, and its loss causes autoimmunity and immune dysregulation. The patient presented to a local hospital with weakness and fatty stool at the age of 10. He had autoimmune hemolytic anemia (AIHA) with splenomegaly and was treated with corticosteroids. Laboratory tests showed panhypogammaglobulinemia. He was referred to a pediatric immunology clinic for further evaluation. He had splenomegaly, failure to thrive, multiple lymphadenopathies, pancytopenia, panhypogammaglobulinemia and *Cryptosporidum* diarrhea. Lymphocyte subsets were normal with poor responses to vaccines, bone marrow aspiration and viral serology were also normal. Lymphadenopathies regressed with antibiotics. Regular intravenous immunoglobulin replacement and prophylactic antibiotics were initiated with the diagnosis of common variable immunodeficiency (CVID). As AIHA was refractory to medical therapy, he underwent splenectomy. During follow-up, he was also treated for short stature and osteoporosis. He was hospitalized several times for fungal or bacterial bronchopneumonia and diarrhea due to *Giardia lamblia* or *Cryptosporidium*. He had lymphoproliferation during infectious episodes. Genetic analysis for X-linked lymphoproliferative syndrome (*SH2D1A* gene) was normal. Computerized tomography of the thorax showed chronic fibrotic lung changes and bronchiectasis. He also had EBV and CMV viremia. We identified a heterozygous mutation in *CTLA4* gene (c.518G>A, p.Gly173Glu) by targeted next-generation sequencing, he was 18 at the time of diagnosis. Many CTLA-4-deficient patients are clinically diagnosed with CVID. As the phenotype of CTLA-4 deficiency includes autoimmunity, recurrent infections and lymphoproliferation, a high level of suspicion in CVID patients is required for earlier diagnosis.

P.B3.02.03

Profiling calcium signals of *in vitro* polarized human effector CD4⁺ T cells

S. Kircher, M. Merino-Wong, B. Niemeyer, ***D. Alansary****;*
Molecular Biophysics, Homburg, Germany.

Differentiation of naïve CD4⁺ T cells into effector subtypes with distinct cytokine profiles and physiological roles is a tightly regulated process, the imbalance of which can lead to an inadequate immune response or autoimmune disease. The crucial role of Ca^{2+} signals, mainly mediated by the store operated Ca^{2+} entry (SOCE) in shaping the immune response is well described. However, it is unclear if human effector CD4⁺ T cell subsets show differential Ca^{2+} signatures in response to different stimulation methods. Herein, we provide optimized *in vitro* culture conditions for polarization of human CD4⁺ effector T cells and characterize their SOCE following both pharmacological store depletion and direct T-cell receptor (TCR) activation. Moreover, we measured whole cell Ca^{2+} release activated Ca^{2+} currents (I_{CRAC}) and investigated whether the observed differences correlate to the expression of CRAC genes. Our results show that Ca^{2+} profiles of helper CD4⁺ Th1, Th2 and Th17 are distinct and in part shaped by the intensity of stimulation. Regulatory T cells (Treg) are unique being the subtype with the most prominent SOCE response. Analysis of *in vivo* differentiated Treg unraveled the role of differential expression of ORAI2 in fine-tuning signals in Treg vs. conventional CD4⁺ T cells.
This work was funded by: HOMFORExcellent (UdS), DFG grants: FOR 2289-P6 and SFB894 A2.

P.B3.02.04

Identification of a key epigenetic barrier towards terminal cytotoxic T lymphocyte differentiation

M. A. ASLAM[1], *M. F. Alemdehy*[1], *E. M. Maliepaard*[1], *T. v. Welsem*[1], *D. v. Dinther*[2], *J. d. Hann*[2], *F. v. Leeuwen*[1], *H. Jacobs*[1];
[1]*The Netherlands Cancer Institute, Amsterdam, Netherlands,* [2]*VU University Medical Center, Amsterdam, Netherlands.*

T cell development and differentiation requires highly specific and tight epigenetic regulation, involving specific alterations to the chromatin structure by covalent modifications of histones. Here we study the role of histone modification, specifically H3K79 methylation in T cell biology. H3K79 methylation is a unique histone mark that requires the activity of the evolutionary conserved methyltransferase DOT1L. H3K79me is associated with transcription and in some cases with the inhibition of repression of the genes it methylates. To accomplish this goal, *DOT1L*was selectively ablated in the T cell lineage at an early developmental stage. DOT1L ablation did not grossly affect intrathymic T cell development. However, transcriptome analysis of single positive (SP) mature CD8$^+$ thymocytes revealed an upregulation of memory associated genes that normally require antigen exposure in the periphery. This effect of *Dot1L* ablation was most striking in the peripheral CD8$^+$ T cells. Here, without any deliberate immunogenic exposure, the virtual absence of naïve CD8$^+$T cells was accompanied with a dramatic increase in memory-like CD8$^+$ T cells. Our results indicate that DOT1L is key element in establishing a barrier towards terminal T cell differentiation. Future studies will address the functional potential of T cells lacking DOT1L as well as the mechanism by which the methylation mark at H3K79 maintains naivety.

P.B3.02.05

Immunomodulatory effects of a soluble form of human CD6 in experimental cancer

S. Casadó-Llombart[1], *I. T. Simões*[1], *M. Velasco-de Andrés*[1], *M. Consuegra-Fernández*[1], *F. Aranda*[1], *E. Carreras*[1], *F. Lozano*[1,2,3];
[1]*Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain,* [2]*Servei d'Immunologia, Centre de Diagnòstic Biomèdic, Hospital Clínic de Barcelona, Barcelona, Spain,* [3]*Departament de Biomedicina, Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain.*

CD6 is a surface receptor expressed by all T cells and a subset of B and NK cells. In T cells, it is physically associated to the TCR/CD3 complex and it mediates cell adhesion and modulation of TCR signaling during T cell activation and differentiation, likely through interactions with its reported ligands (CD166/ALCAM, CD318 and Galectin-1/3). To elucidate the *in vivo* relevance of CD6-mediated interactions, the decoy receptor effects of recombinant soluble human CD6 (rshCD6) were analyzed. Thus, C57BL/6 mice treated with rshCD6 (1.25mg/kg; *i.p.*) every other day for two weeks showed significantly reduced total cell numbers in spleen, increased total cell numbers in peritoneum and decreased percentage of Treg in lymph nodes, compared with vehicle-treated control mice. The functional significance of such changes was evidenced by observation of an enhanced anti-tumoral response when rshCD6-treated mice were simultaneously challenged with melanoma B16-F0 cells (7x10^4 cells, *s.c.*) compared with human seroalbumin-treated controls. This was concomitant with significantly increased NK and decreased Treg percentages in tumor-draining and contralateral lymph nodes. *In vitro* studies showed that rshCD6 decreased Treg but not Th1, Th2 and Th17 polarization of naïve CD4$^+$ T cells in a dose-dependent manner. Taken together, these results support decoying CD6 ligand-receptor interactions by rshCD6 as a feasible strategy for immunomodulation in cancer. Supported by WCR (14-1275), Fundació La Marató TV3 (201319-30), Portuguese FCT (SFRH/BD/75738/2011), and Spanish MINECO (SAF2016-80535-R, BES-2014-069237), ISCIII (SB Program; CD15/00016), and MECD (FPU15/02897), -co-financed by European Development Regional Fund "A way to achieve Europe".

P.B3.02.06

Improved survival in HPV$^+$oropharyngeal cancer patients is associated with tumour-resident CD8$^+$ T cells

R. V. Hewavisenti[1], *A. L. Ferguson*[1], *D. Jones*[2], *A. Hong*[2], *U. Palendira*[1];
[1]*Centenary Institute for Cancer Medicine and Cell Biology, Sydney, Australia,* [2]*Central Clinical School, Royal Prince Alfred Hospital, Sydney, Australia.*

Introduction: Human papilloma virus-positive (HPV$^+$) oropharyngeal squamous cell carcinoma (OSCC) is a clinically and immunologically distinct subset of head and neck cancer, which is increasing in prevalence. HPV$^+$ tumours have shown improved patient prognoses compared to HPV-negative (HPV$^-$) tumours. Tumour-resident CD8$^+$ T cells (TR8s) have been associated with better patient survival in various cancers, but have not been extensively studied in virally-induced cancers. Differing patient outcomes, between HPV$^+$ and HPV$^-$ tumours, illustrate the importance of tailoring treatments to specific subsets of OSCC, which may be altered in intensity based on immunological mechanisms. To understand the role TR8s play in HPV$^+$OSCCs, we investigated whether TR8 numbers correlate with patient survival, in the context of HPV status, tumour microenvironment, and clinical outcomes.
Materials and Methods: Infiltrating TR8s were analysed using quantitative multiplex immunofluorescence staining and multi-parameter flow cytometry, using tumours from a prospective cohort of HPV$^+$OSCC and HPV$^-$OSCC patients.
Results: HPV$^+$OSCC patients had higher TR8 numbers compared to HPV$^-$OSCC patients, with higher infiltration of total CD8$^+$ T cells within HPV$^+$ tumours. Increased TR8 numbers were associated with better patient prognosis and improved survival in HPV$^+$OSCC patients, compared to those with lower TR8 numbers.
Conclusions: The higher TR8 numbers found in HPV$^+$OSCC, compared to HPV$^-$OSCC, suggests that TR8s may be critical in controlling OSCC tumour progression, resulting in better patient prognosis. Further investigation of TR8s may provide a better understanding of how we approach vaccine strategies, with the aim of boosting TR8 numbers and improving the immune response.
Support: The University of Sydney

P.B3.02.07

TCR/ITK signaling tunes CD8+ T cell metabolism, homeostatic proliferation and anti-tumor effector function

W. Huang[1,2], *J. Luo*[3], *A. August*[2,4];
[1]*Department of Pathobiological Sciences, Louisiana State University, Baton Rouge, LA, United States,* [2]*Department of Microbiology and Immunology, Cornell University, Ithaca, NY, United States,* [3]*Department of Biological Chemistry and Neuroscience, Center for Sensory Biology, The Johns Hopkins University School of Medicine, Baltimore, MD, United States,* [4]*Howard Hughes Medical Institute, Cornell University, Ithaca, NY, United States.*

T cell homeostatic proliferation (HP) is regulated by T cell receptor (TCR) signals and homeostatic cytokines, and suggested to be proportional to TCR signal strength. However, we show that ITK, a positive regulator of TCR signaling, negatively tunes CD8$^+$ T cell metabolism, HP and effector function. Under lymphopenic environments, *Itk*$^{-/-}$ CD8$^+$ T cells exhibit T cell-intrinsic, immediate and massive HP, which requires mTOR activity and can be driven by T cell-T cell interaction. TCR signals through ITK to tune IL-7-mediated CD8$^+$ T cell metabolism and HP in an mTOR-dependent manner. The lack of ITK also resulted in enhanced effector cell programming, antigen sensitivity and anti-tumor immunity of the HP cells. Thus TCR signaling via ITK, is a negative tuner of CD8$^+$ T cell homeostasis, metabolism and effector function, and may be a target for clinical benefit in cancer therapy.

P.B3.02.08

BTLA and PD-1 signaling pathways regulate proliferation and cytotoxicity of human V delta 2 gamma/delta T cells independently

H. Hwang[1], *K. Koh*[2], *J. Lee*[1], *S. Kang*[3], *H. Im*[2], *N. Kim*[1];
[1]*Asan Institute for Life Sciences and Department of Convergence Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of,* [2]*Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, Republic of,* [3]*Department of Pediatrics, Korea University Anam Hospital, Seoul, Korea, Republic of.*

B- and T-lymphocyte attenuator (BTLA) and programmed cell death-1 (PD-1) inhibit T cell activation and function through recruitment of SHP2. Although there are studies on the individual roles of BTLA and PD-1 in human gd T cells, it is not well known how these immune checkpoints interact in response to cancer cells. This study was set up to examine whether BTLA and PD-1 signaling pathways were convergent or independent in human peripheral blood gd T cells, where Vg9Vd2 T cells recognizing phospho-antigens are majority. Herein we show that BTLA/HVEM and PD-1/PD-L1 interactions suppressed proliferation and cytotoxicity of human gd T cells, respectively. As expected, IL-2 and zoledronate-mediated proliferation of gd T cells was increased when PBMCs were co-cultured with inactivated HVEM^{low} Jurkat cells, compared with that of wild-type Jurkat cells. CD107a expression and cancer cell death were not affected in expanded gd T cells by co-culture with inactivated HVEM^{low} Jurkat cells and further increased in the presence of anti-PD-L1 mAb. The results suggest that inactivation of BTLA/HVEM signaling pathway during expansion could help produce more gd T cells without compromising cytotoxicity. In addition, reduction of HVEM expression in Jurkat cells repressed phosphorylation of SHP2 and increased activation of ERK1/2 in gd T cells. However, blockade of PD-L1 signaling did not have a synergistic or additive effect on it. Taken together, our study demonstrates that BTLA and PD-1 signaling may independently act on the proliferation, cytotoxicity and phosphorylation of SHP2 and ERK1/2 in human Vd2 gd T cells.

P.B3.02.09

Costimulatory molecule B7-H3 in Myelodysplastic syndrome

A. Kindermann, *P. Mandasari, D. Quandt;*
University of Halle, Halle, Germany.

Costimulation is an important way of regulating T cell immunity. B7-H3 belongs to the B7 family of costimulatory molecules and has been shown to modulate T cell outcomes in health and disease. Many solid tumor cells in situ show a differential expression of B7-H3 that is associated to patient's outcome. The role of B7-H3 for hematological disease is not well established. Myelodysplastic syndrome (MDS) is a hematological stem cell disease leading to diverse phenotypes of altered red and white blood cell hematopoiesis. Analyzes of human bone marrow samples from patients with MDS, other hematological disorders and healthy donors using flow cytometry were performed. B7-H3 expression was found on blast cells, HSC as well as on myeloid cells. In leukemia (THP-1, HL-60, K562) as well as MDS cell lines, B7-H3 protein is expressed constitutively with a great heterogeneity. This is in contrasts to solid tumor cell lines that show a homogenous high level of B7-H3 expression.

Cytokine treatment with important adaptive as well as innate mediators did not alter B7-H3 protein expression, whereas other molecules of the B7 family as well as of the antigen presentation are highly regulated. Co-culture assays of B7-H3 modulated THP-1 cells with human primary T cells are currently performed in order to reveal the functional consequences of this costimulatory pathway. T cells are an important part of the stem cell niche. Therefore we believe on a contribution of costimulatory molecules on the regulation of the complex hematopoietic stem cell network.

P.B3.02.10

Strong sustained IL-2 signal selectively targeted to CD25$^+$ cells dramatically increases sensitivity to LPS

*J. Tomala, P. Weberova, B. Tomalova, **M. Kovar**;*
Institute of Microbiology, Prague, Czech Republic.

IL-2 exerts its pleiotropic activities through binding either to dimeric receptor composed from IL-2Rβ (CD122) and common cytokine receptor gamma chain (γ_c, CD132) or to trimeric receptor composed from IL-2Rα (CD25), IL-2Rβ and γ_c. CD25 has been termed "low-affinity" IL-2R (Kd~10 nM) and it is not involved in signal transduction. A dimer of CD122 and CD132 binds IL-2 with intermediate affinity (Kd~1 nM) and is present on CD122^{high} populations, namely memory CD8$^+$ T cells and NK cells. A complex of CD25, CD122 and CD132 binds IL-2 with high affinity (Kd~10 pM) and it is present on CD25^{high} populations, namely activated T and Treg cells. It was reported that *in vivo* biological activity of IL-2 can be dramatically increased by association of IL-2 with anti-IL-2 mAbs. These IL-2 complexes possess selective stimulatory activity determined by the clone of anti-IL-2 mAb used. IL-2/S4B6 mAb complexes are highly stimulatory for NK and memory CD8$^+$ T cells and intermediately also for Treg cells. IL-2/JES6-1 mAb complexes are stimulatory solely for CD25^{high} cells. We have found that mice treated with IL-2/JES6-1 mAb complexes show dramatically increased sensitivity to LPS-mediated shock and mortality (~10-30 times). Mice treated with IL-2/JES6-1 mAb complexes and challenged with 10 μg LPS possess 5-10 times higher plasma concentration of TNF-α (90 min. after LPS challenge) in comparison to control mice challenged with 200 μg LPS. Interestingly, IL-2/S4B6 complexes almost do not sensitize mice to LPS. **Acknowledgement:** This work was supported by grant 13-12885S from GACR and Institutional Research Concept RVO 61388971.

P.B3.02.11

IFN-g and TNF-a production by T cells in HNSCC stroma promotes a distinct transcriptional signature with immunosuppressive properties by tumor-enriched mesenchymal stem cells

***A. Mazzoni**[1], G. Montaini[1], M. Ramazzotti[1], G. Barra[2], L. Maggi[1], M. Capone[1], M. Rossi[1], B. Rossettini[1], R. De Palma[2], L. Cosmi[1], F. Liotta[1], F. Annunziato[1];*
[1]University of Florence, florence, Italy, [2]University of Campania, Naples, Italy.

Introduction: Mesenchymal stem cells (MSC) are enriched in Head-Neck squamous cell carcinoma (HNSCC) and display immunosuppressive properties, thus favoring tumor immune escape. The identification of the molecular programs involved may suggest novel therapeutical targets. Methods: HNSCC specimens were enzyme-digested. Tumor infiltrating lymphocytes (TIL) were analyzed by flow cytometry to investigate their phenotypic and functional properties. MSC were derived from HNSCC and bone marrow (BM) and expanded *in vitro*. Transcriptome data were generated via microarray technology. Quantitative PCR and flow cytometry were used to validate transcriptome data. T cell proliferation was evaluated via 3H-TdR incorporation assay. Results: We observed an accumulation of IFN-γ and TNF-α producing T cells in HNSCC specimens. T regulatory (Treg) cells were also enriched if compared to peripheral blood (PB). To investigate how IFN-γ and TNF-α affect the immunosuppressive potential of MSC, we obtained transcriptome data from resting or IFNg+TNFa stimulated BM- and HNSCC-MSC. Principal component analysis showed that BM- and HNSCC-derived MSC have a distinct transcriptional signature either resting or after cytokine stimulation, thus suggesting that are distinct cell subsets. Looking for genes selectively induced by cytokine treatment that may confer immunosuppressive potential to MSC we found IDO1, PD-L1 (CD274) and IL4I1. We confirmed microarray data by qPCR or flow cytometry. Selective inhibition of these three pathways during In vitro MSC-T cell cocultures highlighted their immunosuppressive capacities. Conclusions: HNSCC-TIL are enriched in Treg cells, while T effector cells-derived IFN-γ and TNF-α potentiate MSC immunosuppression via activating several non-redundant pathways. Altogether, these mechanisms favor HNSCC immune-evasion.

P.B3.02.12

Tim-3$^+$ CD4$^+$ T cells in the breast tumor draining lymph nodes

***F. Mehdipour**[1], S. Shariati[1], A. Ghods[1], A. Talei[2], A. Ghaderi[1];*
[1]Shiraz Institute for Cancer Reaserch, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of, [2]Breast Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of.

Introduction: It has been suggested that T cell immunoglobulin and mucin domain-3 (Tim-3) is an important immune checkpoint receptor which can be involved in the immune suppression in the tumor microenvironment. It has been shown that exhausted or regulatory T cells express Tim-3. In this study, the frequency of Tim-3$^+$CD4$^+$T cells in the tumor draining lymph nodes (TDLNs) of breast cancer patients and its relation with disease parameters were investigated.
Materials and methods: Using Ficoll-Hypaque gradient centrifugation, mononuclear cells were isolated from axillary lymph node specimens of 35 breast cancer patients. After surface staining for CD4 and Tim-3, cells were subjected to flow cytometry.
Results: Our results revealed that 6.4± 5.8% of CD4$^+$ T cells expressed Tim-3 without significant difference in the metastatic (MLNs) and non-metastatic lymph nodes (nMLNs). The mean fluorescent intensity (MFI) of Tim-3 expression showed a non-significant increasing trend in the MLNs (P=0.091), however when considering patients with invasive ductal carcinoma and excluding patients whose tumor had medullary features, the MFI of Tim-3 was significantly higher in the MLNs (P=0.023). In addition, the MFI of Tim-3 expression had significant direct correlation with the number of involved lymph nodes (R=0.3, P=0.041). The frequency of Tim-3 expressing T cells or Tim-3 MFI did not show significant associations with stage, grade or tumor size.
Conclusion: This study revealed that a fraction of CD4$^+$ T cells expressed Tim-3 in the TDLNs of breast cancer patients. Lymph node involvement was associated with higher intensities of tim-3 expression on T cells.

P.B3.02.13

Conserved human effector regulatory T cell signature is reflected in super-enhancer landscape

***G. Mijnheer**[1], M. Mokry[1], V. Fleskens[2], M. van der Wal[1], R. Scholman[1], S. Vervoort[1], C. Roberts[2], A. Petrelli[1], J. Peeters[1], M. Knijff[1], S. de Roock[1], S. Vastert[1], L. Taams[2], J. van Loosdregt[1], F. van Wijk[1];*
[1]University Medical Center Utrecht, Utrecht, Netherlands, [2]King's College London, London, United Kingdom.

Regulatory T cells (Treg) are critical regulators of immune homeostasis. Increasing evidence demonstrates that environment-driven Treg differentiation into effector Treg is crucial for optimal functioning. However, programming of human Treg under inflammatory conditions remains poorly understood. Here, we combine transcriptional and epigenetic profiling to identify the human effector Treg core signature. Autoimmune inflammation-derived Treg demonstrated normal suppressive function and enhanced IL-2 signaling. Transcriptome analysis revealed a unique transcriptional profile characterized by upregulation of both a core Treg (FOXP3, CTLA-4, TIGIT) and effector program (ICOS, GITR, BLIMP-1, BATF, T-bet), indicating effector Treg differentiation and adaptation to the inflammatory environment. Moreover, we identified specific human effector Treg markers including VDR and IL12Rβ2. H3K27ac occupancy revealed large changes in the (super-)enhancer landscape, including enrichment of the binding motif for VDR and BATF. The observed Treg profile showed striking overlap with tumor-infiltrating Treg. Our data demonstrate that human inflammation-derived Treg acquire a specific effector Treg profile guided by epigenetic changes. The core effector Treg profile is strongly conserved, and fine-tuned by environment specific adaptations.

P.B3.02.14

Type 1 regulatory cells (Tr1) express c-MAF in response to CD55 but not CD28 costimulation

***T. Musarrat**;*
University of Nottingham, Nottingham, United Kingdom.

A healthy immune system is maintained in a state of balance between pro-and anti-inflammatory cells. The paradigm for T-cell activation requires CD80/86:CD28 engagement resulting in differentiation of proinflammatory response. However, alternative costimulatory molecules may favour the induction of alternate T-cell phenotypes such as Type 1 Regulatory T-cells (Tr1). One such receptor-ligand pair is CD55-CD97. These are widely expressed on leukocytes, including T cells, dendritic cells (DC) and macrophages. We have previously demonstrated that costimulation of T cells via CD3/CD55 results in the differentiation of naive T-cells into Tr1 phenotype which is defined as IL-10$^+$, IFN-γ$^-$ IL-4$^-$ as opposed to Th1 phenotype (IFN-γ$^+$, IL-10$^-$, IL-4$^-$) by CD3/CD28 costimulation. IL-10 is the predominant inhibitory cytokine produced by adaptive immune system and it is required for immune resolution, promoting tolerance and controlling autoimmunity. Considering the important role of IL-10$^+$ Tr1 in immune-balance, there still remains little in the way of a defined phenotype for these cells. We aimed to study CD3/CD55 induced Tr1 cells in order to determine the transcription factors associated with the regulation of these cells. CD3/CD55 induced IL-10$^+$ Tr1 cells were positive for T-bet and c-MAF whereas they were negative for FoxP3, GATA-3 and HELIOS. Interestingly, c-MAF was only expressed by IL-10$^+$ cells in response to CD3/CD55 but not to CD3/CD28 stimulation. c-MAF expression was persistent upon secondary restimulation with CD3/CD55 and was not induced by non-specific stimulation with PMA/Ionomycin indicating that c-MAF expression could be an integral part of signalling for CD3/CD55 mediated IL-10 production by Tr1 cells.

P.B3.02.15

Comparison of glucocorticoid-induced apoptosis in thymocytes and mature T cells

***L. Prenek**, R. Kugyelka, F. Boldizsár, P. Németh, T. Berki;*
Department of Immunology and Biotechnology, Pécs, Hungary.

Non-genomic effects of the glucocorticoids (GC) play an important role in the GC-induced apoptosis of thymocytes. We have found that in the most GC sensitive, DP thymocytes the activated GC receptor (GR) translocated to the mitochondria, which was followed by the decrease of the mitochondrial membrane potential, an early sign of apoptosis. The activated GR associated with members of the Bcl-2 protein family, especially with Bim, and influenced their balance leading to the activation of mitochondrial apoptotic pathway. The effect of *in vivo* dexamethasone (DX) treatment on regulatory T cells (Tregs) was different; they seemed to be resistant to DX-induced apoptosis. Which suggests that the GC-induced apoptotic process is different in other cell populations. But the exact mechanism requires further investigation. In our work we analysed the differences and similarities between the DX-induced apoptosis of thymocytes and mature T cells, especially that of Tregs. Splenocytes and thymocytes were isolated from 4-6-week-old BALB/c mice. The cells were treated with 10^{-6} M DX *in vitro* for different intervals. After DX treatments Annexin V labelling, kinetics of caspases' activation, mitochondrial membrane potential and Ca-signalling were analysed with flow cytometry. DX-induced apoptotic process was different in mature T cells, particularly in Tregs, than in thymocytes. DX inhibited Ca-signalling of cell populations suggesting its potential role in apoptotic signalling. These results can be the consequences of the different non-genomic effects in T cell subpopulations. Funding: OTKA K105962, K101493, EFOP-3.6.1.-16-2016-00004; GINOP 2.3.2-15-2016-00050.

P.B3.02.16

The role of Ca^{2+} dependent proteins for the killing capacity of human cytotoxic T cells

***S. Zöphel**[1], G. Schwär[1], P. Leidinger[2], K. S. Friedmann[1], A. Knörck[1], C. Hoxha[1], E. Meese[2], V. Helms[3], M. Hoth[1], M. Hamed[4], E. C. Schwarz[1];*
[1]Biophysics, Homburg, Germany, [2]Human Genetics, Homburg, Germany, [3]Center for Bioinformatics, Saarbrücken, Germany, [4]Institute for Biostatistics and Informatics in Medicine and Ageing Research, Rostock, Germany.

Introduction: Cytotoxic T lymphocytes (CTLs) eliminate infected or transformed cells by releasing perforin-containing cytotoxic granules at the immunological synapse. Cytotoxic granule release is highly dependent on the influx of extracellular Ca^{2+}, mediated by STIM-activated Orai channels. Previous work has shown that members of the SNARE-family are primarily responsible for exocytosis of lytic granules. However, considerably less is known about other molecules modulating the Ca^{2+} influx and exocytosis of cytotoxic granules, and thereby elimination of target cells. **Material and Methods**: Human PBMC were isolated from LRS chambers provided by the local blood bank. Subtype isolation was performed by antibody-coated magnetic beads. Total RNA from non-stimulated $CD8^+$ T cells or from *Staphylococcus aureus* enterotoxin A (SEA) stimulated $CD8^+$ T cells was isolated and investigated by microarray technology. Expression data were further analyzed by a bioinformatical approach. **Results:** Based on the results of the bioinformatics analysis, we selected 42 siRNAs against highly expressed proteins with a potential function in calcium signaling pathways and screened for a putative role in target cell killing. By using a real-time killing assay under reduced extracellular Ca^{2+} concentrations, we identified several candidates which altered the killing capacity of $CD8^+$ T cells. One candidate is the Ca^{2+}-induced potassium channel KCa3.1. Its downregulation decreased the elimination of target cells under limited extracellular Ca^{2+} concentration. **Conclusion:** Bioinformatical analysis is a powerful tool to narrow-down possible candidates relevant for target elimination by CTL. Screening of the selected siRNAs by a real-time killing assay led to positive hits under limited extracellular Ca^{2+} concentration.

P.B3.02.17

Engineering chimeric antigen receptor T cells to specifically target *Aspergillus fumigatus*

***M. Seif**[1], T. Nerreter[1], M. Machwirth[1], M. Hudecek[1], F. Ebel[2], H. Einsele[1], J. Löffler[1];*
[1]University Hospital Wuerzburg, Wuerzburg, Germany, [2]Ludwig-Maximilians-University, Munich, Germany.

Immunocompromised patients are susceptible to invasive fungal infections mainly caused by *Aspergillus fumigatus* (Af). Adoptive transfer of *Aspergillus*-specific T cells reduces the burden of invasive aspergillosis. Such specific T cells are hard to isolate and expand. Alternatively, T cells modified to express a chimeric antigen receptor (CAR) can be used. CARs are recombinant receptor constructs composed of an extracellular single-chain antibody fragment (scFv) linked to an intracellular signaling module.
To redirect specificity towards Af, a scFv derived from an antibody directed against Hyphal cell wall was designed. CARs containing the scFv fused to extracellular IgG4-Fc spacer domains of different lengths were constructed. T cells were engineered to express the CARs on their surface using the Sleeping Beauty gene transfer system. CAR T cells were co-cultured with Af germ tubes and specific T cell activation was evaluated.
Upon binding to the target, CAR T cells signaled via chimeric CD28 and CD3-ζ signaling domain. The cytolytic machinery of CAR T cells was activated leading to the release of perforin and granzyme B. Activated CAR T cells secreted cytokines like IFN-γ and IL-2. Furthermore, activated CAR T cells underwent proliferation. Finally, CARs containing long "Hinge-CH2-CH3" extracellular spacer conferred superior T cell activation when compared with CARs having short "Hinge-only" spacer.
Our results show that CAR T cells are specially activated upon recognition of Af and customizing spacer design enhances their effector function.
This project is funded by the BMBF (Infect Control 2020—consortium ART4Fun; subproject 2 to HE).

P.B3.03 T-cell regulation - Part 3

P.B3.03.01

Human memory CD8 T cell effector function is epigenetically preserved during in vivo homeostasis

***H. Abdelsamed**[1], A. Moustaki[1], Y. Fan[1], P. Dogra[1], H. Ghoneim[1], C. Zebley[1], B. Triplett[1], R. Sekaly[2], B. Youngblood[1];*
[1]St Jude Children's Research Hospital, Memphis, United States, [2]Case western Reserve University, Cleveland, United States.

Maintenance of memory CD8 T cell quantity and quality through antigen-independent homeostatic proliferation is vital for sustaining long-lived T cell-mediated immunity, yet the underlying mechanisms that preserve memory T cell functions during homeostasis remain largely unexplored. Here we show that preservation of effector-potential among human memory CD8 T cells during *in vitro* and *in vivo* homeostasis is coupled to maintenance of memory-associated DNA methylation programs. Whole-genome bisulfite sequencing of primary human naïve, short-lived effector memory (T_{em}), and longer-lived central memory (T_{cm}) and stem cell memory (T_{scm}) CD8 T cells identified demethylated promoters of effector molecules that are poised for rapid expression among all memory cell subsets. Effector-loci demethylation was heritably preserved during IL-7 and IL-15 mediated *in vitro* cell proliferation. In contrast to the effector-potential, antigen-independent proliferation induced a phenotypic conversion of T_{cm} and T_{scm} memory cells into T_{em} cells that was coupled to increased methylation of the CCR7 locus. Furthermore, *in vivo* proliferation of haploidentical donor memory CD8 T cells in lymhodepleted recipients resulted in a similar preservation of effector-associated methylation programs while enriching for Tem-associated programs. These data demonstrate that long-lived human memory CD8 T cells retain the ability to undergo antigen-independent epigenetic reprogramming during their developmental conversion into other memory subsets while at the same time preserving the poised effector state utilized by all memory T cells. Further investigation into upstream signaling events that promote changes in T cell epigenetic states is needed to uncover the role of epigenetics in T cell function during homeostasis.

P.B3.03.02

Tissue-resident memory T-lymphocytes (T_{RM}) display proliferative capacity *in vitro* under hypoxic conditions

***A. Chuwonpad**[1], F. M. Behr[1,2], T. H. Wesselink[1], N. A. Kragten[1], R. Stark[1,2], K. P. van Gisbergen[1,2];*
[1]Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands, [2]Academic Medical Center, Amsterdam, Netherlands.

Tissue-resident memory (T_{RM}) have been recently established as an important subset of memory cells that provide early and essential protection against reinfection in the absence of circulating memory cells. In addition, $CD8^+$ T cells with a T_{RM}-like phenotype have been found to infiltrate tumor tissue. The presence of these T_{RM}-like cells are associated with patient survival, suggesting that T_{RM} also have a protective role in cancer. Immunotherapy using adoptive transfer of *in vitro* reactivated T cells from the tumor site has provided a breakthrough in the treatment of cancer patients. Despite promising results, further improvement of therapy is required for the majority of patients. Because T_{RM} are beneficial in both cancer and infection settings, we aim to study whether these memory T cells can be expanded in culture for immunotherapy-related purposes.
We have found that T_{RM} from the small intestine proliferate *in vitro* after anti-CD3/28 stimulation and subsequent resting in cytokines. The expanded T_{RM} retained their phenotype, including expression of key T_{RM} markers CD69 and CD103. Optimal culture of T_{RM} required low O_2 tension, indicating that these cells can resist hypoxic conditions in the tumor microenvironment. T_{RM} also exerted increased metabolic activity and efficiently acquired glucose, a requirement to compete with highly glycolytic tumor cells.
These findings suggest that *in vitro* re-stimulation of existing T_{RM} is possible under conditions reflecting the tumor microenvironment, which is promising for future usage of these memory T cells in tumor treatment using adoptive cell therapy.

P.B3.03.03

Dissecting the role of MAZR isoforms in T cell development and function

***N. Dhele**, L. Anderson, A. Gülich, S. Sakaguchi, W. Ellmeier;*
Medical University of Vienna, Vienna, Austria.

T cells are key players of adaptive immunity and their differentiation and function has been tightly controlled. We previously identified that the transcription factor MAZR, also known as Patz1, is an important regulator of *Cd8* gene expression during the double-negative (DN) to double-positive (DP) transition of T cell development. Further, by generating MAZR-deficient mice we showed that MAZR is part of the transcription factor network regulated CD4/CD8 cell fate decision of DP thymocytes. MAZR is a member of the BTB domain and zinc finger (ZF) motifs containing transcription factor family and is encoded by the *Patz1* gene. There are 4 potential alternative splice forms known that can give rise to MAZR isoforms containing 4-7 ZF domains, however whether all splice forms are expressed in T cells has not been described. Moreover, the role of the various MAZR isoforms in the regulation of T cell development and function is not known. The aim of the study is to dissect isoform- specific functions of MAZR by using gain-of-function and loss-of-function approaches in combination with RNA-seq and ChIP-seq experiment. Results from ongoing studies will be presented. The project is funded by the EU ITN Grant “ENLIGHT-TEN” (675395) and the Austrian Science Fund (P29790)

P.B3.03.04

Regulation of integrin activation on antigen-specific T cells by Galpha$_s$-coupled receptor signaling and sleep in humans

***S. Dimitrov**[1], T. Lange[2], C. Gouttefangeas[1], A. T. Jensen[3], M. Szczepanski[1], J. Lehnnolz[1], S. Soekadar[1], H. Rammensee[1], J. Born[1], L. Besedovsky[1];*
[1]University of Tübingen, Tübingen, Germany, [2]University of Lübeck, Lübeck, Germany, [3]University of Copenhagen, Copenhagen, Denmark.

An efficient T-cell effector immune response requires a strong adhesion of T cells to their targets, e.g., to virus-infected cells. This adhesion is dependent on the immediate activation of β_2-integrins upon T-cell receptor (TCR) engagement by cognate peptides presented by major histocompatibility complex molecules (pMHC). Gα_s-coupled receptor agonists are known to have immunosuppressive effects, but their impact on TCR-mediated integrin activation is unknown so far. We used soluble multimers of pMHC and ICAM-1 - the ligand of β_2-integrins - to assess the effect of different Gα_s-coupled receptor agonists (including catecholamines, prostaglandins (PGs), adenosine, histamine and serotonin) on TCR-induced integrin activation of human cytomegalovirus (CMV)- and Epstein-Barr (EBV) virus-specific CD8$^+$ T cells. We show that isoproterenol, epinephrine, norepinephrine, PGE_2, PGD_2 and adenosine strongly inhibit TCR-mediated integrin activation of antigen-specific CD8$^+$ T cells in a dose-dependent manner, already at physiological concentrations. Using sleep as a natural condition of low levels of Gα_s-coupled receptor agonists, we found that nocturnal sleep upregulates TCR-induced integrin activation compared to nocturnal wakefulness. The effect was slightly more pronounced for CMV-specific T cells with an early-differentiated phenotype (CD27$^+$CD28$^+$) than for intermediate- or late-differentiated subsets. Our findings indicate that conditions characterized by low levels of Gα_s-coupled receptors agonists, such as sleep, support the formation of immunological synapses and thereby enhance T-cell responses. The results might be also relevant to a variety of pathologies associated with increased levels of catecholamines (during chronic stress or sleep disturbances), PGs (tumor growth or malaria) and adenosine (hypoxia, sleep apnea, or tumor growth).

P.B3.03.05

LAT transmembrane domain in the plasma membrane of T cells

***D. Glatzová**[1,2], T. Chum[1], J. Králová[2], L. Cwiklik[1], T. Brdička[2], M. Cebecauer[1];*
[1]J. Heyrovsky Institute of Physical Chemistry, Czech Academy of Sciences, Prague, Czech Republic, [2]Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic.

LAT is transmembrane adaptor protein essential for T cell development and function. It is phosphorylated by ZAP70 protein tyrosine kinase upon T cell receptor activation and recruits multiple adaptor proteins to form a multisubunit signalling complex. It contains a very short extracellular region, transmembrane domain (TMD) and tyrosine-rich cytoplasmic tail. LAT is palmitoylated on two conserved cysteines (amino acids 26 and 29). Furthermore, TMD of LAT contains two proline amino acids (position 8 and 17) function of which is still unknown. In agreement with the literature about other proteins, our MD simulations of LAT TMD demonstrate that the presence of prolines disrupts α-helical structure and causes so-called proline-kinks in its structure. Expression of mutants with prolines changed to alanines or in combination with mutants lacking the palmitoylation sites suggest their importance for sorting of LAT to the plasma membrane and their impact on T cell signalling.
Acknowledgement: We would like to acknowledge funding by GA UK project no. 298216. This work was also supported by Czech Science Foundation (15-06989S)

P.B3.03.06

Human double-negative regulatory T cells induce a metabolic switch in effector T cells by suppressing mTOR activity

***T. Haug**, M. Aigner, H. Bruns, A. Mackensen, S. Völkl;*
Dept. of Internal Medicine 5 – Hematology/Oncology, Erlangen, Germany.

The recently discovered subpopulation of TCRαβ+ CD4-/CD8- (double-negative, DN) T cells are highly potent suppressor cells in mice and human. In murine transplantation models, adoptive transfer of DN T cells specifically inhibits alloreactive T cells and prevents development of transplant rejection or Graft-versus-Host-disease (GvHD). Interestingly, clinical studies in patients who underwent stem cell transplantation reveal an inverse correlation between the frequency of circulating DN T cells and the severity of GvHD, suggesting a therapeutic potential of human DN T cells. Investigating the impact of DN T cells on effector T cells, we show that DN T cells diminish upregulation of glycolytic machinery, expression of glucose transporters and glucose uptake. In contrast, the uptake of fatty acids stays unchanged, indicating that DN T cells induce a metabolic switch in effector T cells. Consistent with this finding, DN T cells selectively inhibit the metabolic key regulator mTOR and enforced activation of mTOR in effector T cells with a chemical activator reversed the suppressive effect of DN T cells. Given that both, mTOR activity and cell metabolism are crucial in determining T cell fate, suppressed effector T cell produce less inflammatory cytokines. Taken together DN T cells impair metabolic reprogramming of effector T cells by abrogating mTOR signaling, thereby inducing a quiescent phenotype. These results uncover a new manner of DN T cell mediated-suppression and deeper understanding of DN T-cells may have important implications for using them as a cellular-based therapy to limit alloreactive immune responses.

P.B3.03.07

Rolling circle translation of a circular RNA in T helper lymphocytes

***G. A. Heinz**[1], C. L. Tran[1], P. Durek[1], F. Heinrich[1], Z. Fang[1], C. Haftmann[1], V. Treffner[1], W. Chen[2], H. D. Chang[1], A. Ostareck-Lederer[3], D. H. Ostareck[3], A. Radbruch[1], M. F. Mashreghi[1];*
[1]Deutsches Rheuma-Forschungszentrum, Berlin, Germany, [2]Max Delbrück Center for Molecular Medicine, Berlin, Germany, [3]Medical Faculty, RWTH Aachen University, Aachen, Germany.

Circular RNAs (circRNAs) have gained considerable interest in the course of their re-characterization as endogenous RNA species which is present in various cell types including T cells. However, the function and biological impact of circRNAs on cellular mechanisms still remain poorly understood. Analyzing total RNA sequencing data from naive and activated murine T helper lymphocytes under Th1, Th2 and Th17 polarizing condition, we discovered a highly abundant circRNA (circ11607907). This circRNA candidate showed high expression in naive T helper cells, which strongly decreases with T cell activation and is further reduced upon repeated activation of Th1 cells. The corresponding mRNA expression follows a similar pattern, yet with a stable intermediate expression in Th1 cells. Interestingly, we identified one transcript variant in Th1 cells that harbors an alternative exon 1 and resembles the drastically reduced expression upon T cell activation. This suggests that the circ11607907 might be generated from the alternative transcript rather than from the protein coding mRNA. Dissecting the circ11607907 sequence, we found that it contains an infinite open reading frame with a potential for rolling circle translation. Indeed, ectopic overexpression of the circRNA in murine embryonic fibroblasts resulted in the expression of cryptic proteins which could be abolished by mutating the start codon or introducing a stop codon. Using polysome profiling revealed that the endogenous circ11607907 can be detected in the polysomal fractions indicating that it is also subjected to rolling circle translation.Currently, we aim at deciphering the role of these cryptic proteins in T helper cells.

P.B3.03.08

Development of gene knock-down method in primary T cells

***J. Hillis**, S. Kidger;*
CRUK-TDL, Cambridge, United Kingdom.

Genetic manipulation is an important tool in target validation. Through knock-down and overexpression of genes we can determine effects on biomarkers, determine synthetic lethalities and investigate phenotypic effects. In addition, use of mutated constructs can illuminate the function of protein domains.
Genetic manipulation of primary immune cells is known to be challenging. These cells often have very specific culture conditions required for survival and proliferation, and can be quite sensitive to additional reagents. In addition to this, primary immune cells do not easily take up lipid particles or extracellular DNA. These characteristics make them particularly difficult to genetically manipulate by common methods such as transfection with lipid particles.
We have employed electroporation as a method of delivery of siRNA into primary T cells. Using this method, we can achieve consistent knock-down of at least 70% at both mRNA and protein levels, whilst maintaining good viability. So far, this method has been optimised for use in activated CD3+, CD8+ and expanded Treg populations.
Ongoing work will expand this knock-down method to further T cell subtypes, including unstimulated cells, to allow for wider target validation work. We also intend to develop a robust method of overexpression in primary T cells, which would allow us to investigate protein domain function.

P.B3.03.09

Elevated activation of CD83-deficientT cells

***K. Liedtke**, J. Buer, W. Hansen;*
Institute of Medical Microbiology, Essen, Germany.

The glycoprotein CD83 is upregulated on T cells upon activation. By using a T cell specific CD83 conditional knockout mouse ($CD83^{flx/flx}$/CD4cre, CD83cKO) we aim to define the role of CD83 on T cells. Among the murine T cell subsets CD83 is more frequently expressed on $CD4^+CD25^+$ regulatory T cells (Tregs) in contrast to $CD4^+CD25^-$ conventional T cells (Tcons). Despite the expression on Tregs the inhibitory activity of sorted Tregs and the differentiation of Tcons into induced Tregs was similar between cells from WT and CD83cKO mice in vitro. However, Tcons from CD83cKO mice exhibited a more activated phenotype and showed increased differentiation into T helper 1 cells upon stimulation in vitro. In vivo, CD83cKO mice showed an elevated inflammatory immune response in a contact hypersensitivity reaction compared to WT littermates, which was accompanied with stronger $CD4^+$ T cell activation and secretion of inflammatory cytokines within the spleen and draining lymph nodes. Overall our data indicates that CD83 expression on T cells somehow regulates their activation resulting in an altered disease outcome. Whether the increased activation of CD83-deficient T cells might have a beneficial effect on anti-tumor immune responses will be analyzed in a subcutaneous murine tumor model in vivo.

P.B3.03.10

α-ketoglutarate regulates the balance between Th1 and induced regulatory T cell differentiation

***M. I. Matias**[1], C. S. Yong[1], A. Foroushani[2], M. Wong[2], A. Talebi[3], J. Dehairs[3], C. Mongellaz[1], J. V. Swinnen[3], S. A. Muljo[2], V. Dardalhon[1], T. Naomi[1];*
[1]Institut de Génétique Moléculaire de Montpellier, CNRS, Montpellier, France, [2]Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892, United States, [3]Department of Oncology, KU Leuven, Leuven 3000, Belgium.

The proliferation and differentiation of T lymphocytes is regulated by antigen and cytokine signals but the induction of metabolic pathways is also required, resulting in an integration of environmental cues in cell fate decisions. Indeed, T lymphocyte activation is regulated by the metabolism of glucose, fatty acids and amino acids, allowing the cell to meet increased energetic and biosynthetic demands. Notably, we recently found that glutamine availability is a key determinant of T cell differentiation; CD4 lymphocytes undergo differentiation to IFNγ-secreting Th1 effectors under conditions of optimal glutaminolysis but are converted to $Foxp3^+$ regulatory T cells when glutamine catabolism is abrogated. We now show that the glutamine-derived metabolite, α-ketoglutarate (αKG), significantly augments IFNγ-secretion while attenuating Treg generation. Interestingly, adding αKG increases extracellular nutrients transport and alters human Treg differentiation potential. Mechanistically, αKG inhibited Foxp3 expression via an IFNγ-regulated mechanism and an altered lipid metabolism. Furthermore, αKG promoted the effector activity of chimeric antigen receptor (CAR)-engineered T cells against the HER2 tumor antigen, increasing IFNγ secretion by >10-fold. Thus, our study identifies the potential of a single metabolite to translate metabolic signals into an enhanced anti-tumor CAR-T cell response.

P.B3.03.11

Fatty acid metabolic reprogramming via mTOR-mediated induction of PPARγ directs anabolic requirements in early activation of T cells

***A. Mulki**, Y. Endo, H. K. Asou, T. Yamamoto, D. J. Tumes, H. Tokuyama, K. Yokote, T. Nakayama;*
Department of Immunology, Chiba University Japan, Chiba city, Japan.

To fulfill the bioenergetic and biosynthetic requirements for increased cell size and massive clonal expansion, activated CD4+ T cells need to reprogram their metabolic signatures from energetically quiescent to activated. However, the molecular mechanisms and essential components controlling metabolic reprogramming in T cells remain poorly understood. We herein show that the mTORC1-PPARγ pathway is crucial for the fatty acid uptake program in activated CD4+ T cells. The mTORC1- SREBP1 pathway was also important for the induction of fatty acid biosynthesis program. Both pathways were required for full activation and rapid proliferation of naive and memory CD4+ T cells. PPARγ directly binds and induces genes associated with fatty acid uptake in naïve and memory CD4+ T cells in both mice and humans. The PPARγ-dependent fatty acid uptake program is critical for metabolic reprogramming. Thus, the results of this study provide important mechanistic insight into the metabolic reprogramming mechanisms that govern the expression of key enzymes, fatty acid metabolism and the acquisition of an activated phenotype via the metabolic reprogramming during CD4+ T cell activation.

P.B3.03.12

T cell receptor signal responsive Pellino 1 ubiquitin ligase in monitoring the pathogenesis

***J. Park**, C. Lee;*
Sungkyunkwan University, Suwon, Korea, Republic of.

Ubiquitination has been implicated in many dynamic cellular processes, including transcriptional regulation, regulation of protein-protein interactions and association with ubiquitin-binding scaffolds, and thus the defects within these pathways cause a wide range of diseases. Pellino 1 (Peli1) is initially characterized as a family of ubiquitin E3 ligases that can catalyse the ubiquitination of several proteins involved in the regulation of innate and adaptive immune responses. Importantly, Peli1 is a receptor-mediated signal responsive ubiquitin ligase that promotes the lysine 48- or 63-linked ubiquitination of target substrates.
Ligand-independent activation of receptor-mediated signaling is one of critical mediators for triggering pathogenic processes. Interestingly, the expression of Peli1 protein is highly suppressed under normal and non-pathogenic conditions, and is distinctly activated in response to the various receptor-mediated signalings. We have recently found that the constitutive expression of Peli1 resulted in ligand-independent hyperactivation of B cells and facilitated the development of lymphoid tumors, with prominent B cell infiltration observed across multiple organs. Aberrant regulation of Peli1 expression is also associated with autoimmunity. To examine the gain- or loss-of-functional role of Peli1 in autoimmunity and tumor immunity, we employed both inducible transgenic and T cell specific knockout mouse models. Thus, this study will include the immunologic basis of Peli1-mediated T cell regulation and the related pathogenesis.

P.B3.03.13

Analysis of mitochondrial content and mitochondrial membrane potential in CD8+ T cells from lung cancer patients

***H. Prado-Garcia**, S. Romero-Garcia, R. Sandoval-Martinez, J. S. Lopez-Gonzalez, U. Rumbo-Nava;*
National Institute of Respiratory Diseases, Mexico City, Mexico.

Background: Nutrient supply and energy production are key requirements so that T-cells can proliferate and mediate effector functions. Mitochondrial metabolism has an essential role in activation of CD8+ T-cells. Patients with advanced stages of lung cancer frequently show malignant pleural effusions, which are infiltrated by lymphocytes and tumor cells. Pleural effusion CD8+ T-cells from lung cancer patients show reduced effector functions. We evaluated whether CD8+ T-cells from lung cancer patients have alterations in their mitochondria. Material and Methods: Mitochondrial mass and mitochondrial membrane potential (MMP) were analyzed using specific fluorescent probes in CD8+ T-cells from pleural effusions and peripheral blood of lung cancer patients by multiparametric flow cytometry. For comparison pleural effusions from chronic diseases (tuberculosis) and peripheral blood from healthy donors were included. Results: Mitochondrial mass, measured as mean fluorescence intensity (MFI) of MitoTracker Green, was reduced in pleural effusion CD8+ T-cells respect to CD8+ T-cells from the comparison groups, this phenomenon was accentuated in the memory subset. MMP was decreased (MFI values of $DiOC_6(3)$) in memory CD8+ T-cells from malignant effusions, compared to the same subset from peripheral blood. After polyclonal stimulation, MMP was reduced in memory CD8+ T-cells from lung cancer patients, which correlated with a reduced production of reactive oxygen species. These phenomena were not observed in the other groups. Conclusions: Pleural effusion CD8+ T-cells from lung cancer patients showed increased mitochondrial mass associated to depolarization of mitochondrial membrane potential, which might compromise the antitumor function of CD8+ T-cells.

P.B3.03.14

Innate-likeactivation of memory $CD4^+$ T cells during heterologous challenge with*Legionella pneumophila*

***N. Rakebrandt**, K. Littringer, N. Joller;*
Institute of Experimental Immunology, University of Zürich, Zürich, Switzerland.

Introduction: Several studies have shown that T cells can function as innate sensors and produce cytokines in response to an inflammatory environment in an antigen-independent manner. This bystander activation has mainly been described for memory $CD8^+$ T cells and is often associated with cytotoxicity and tissue damage. Since antigen-independent activation of $CD4^+$ T cells has been described by some studies but is less well understood we aim to shed more light on this phenomenon in the context of a heterologous challenge. Materials and Methods: B6 mice were infected with an acute dose of the Lymphocytic choriomeningitis virus (LCMV) WE and subsequently challenged with *Legionella pneumophila*. The bacterial titer was determined and T cell populations were analyzed by flow cytometry or sorted for further experiments. Results: We show that previous experience of an acute LCMV infection in mice enables antigen-independent protection against a secondary challenge with *L. pneumophila* by allowing faster bacterial clearance. In this setting not only $CD8^+$ T cells but interestingly also $CD4^+$ T cells in the lungs of LCMV-primed mice rapidly produce IFNγ upon heterologous challenge. Similarly to earlier reports for $CD8^+$ T cells, a subset of LCMV-experienced $CD4^+$ T cells can produce IFNγ in response to proinflammatory cytokines in the absence of TCR signaling. Conclusions: Our results indicate that infection-experienced $CD8^+$ as well as $CD4^+$ T cells might help to convey protection against a heterologous pathogen challenge. These long-lived cells harbor the potential for directed and transient activation by cytokine signaling or receptor engagement. Funding: SNF

P.B3.03.15

Circular RNAs are abundantly expressed in a subset-specific manner in T helper lymphocytes

C. L. Tran[1], *G. A. Heinz*[1], *P. Durek*[1], *F. Heinrich*[1], *Z. Fang*[1], *K. Lehmann*[1], *D. Schulz*[1], *C. Haftmann*[1], *W. Chen*[2], *H. D. Chang*[1], *A. Radbruch*[1], *M. F. Mashreghi*[1];
[1]Deutsches Rheuma-Forschungszentrum, Berlin, Germany, [2]Max-Delbrück-Centrum for Molecular Medicine, Berlin, Germany.

Circular RNAs represent a class of covalently closed RNA molecules, which are spliced from primary transcripts in a head-to-tail fashion and generally considered to be non-coding. Due to their ring-structure, they lack both 5' cap and poly-A tail and display exonuclease resistance. Next-Generation Sequencing (NGS) data revealed that circRNAs are abundant and display development- and stage-specific expression patterns, although little is known about the function of most identified circRNAs.
Here, we hypothesize that circRNAs play a role in the function of proinflammatory T helper (Th) cells, which are involved in the initiation and maintenance of chronic inflammatory diseases.
Using NGS, we assessed the global expression profile of circRNAs in naive, once and repeatedly activated Th cells, mimicking protective and proinflammatory Th cells, respectively. As a result, 41,169 potential circRNAs were detected in Th cells. Of the highly expressed circRNAs (>50 reads), the majority consists of one to three exons with an overall enrichment for translation start sites and is largely expressed in all Th subsets with the highest number in naive Th cells. However, their expression is subset- and activation-dependent with overall higher expression in naive Th cells. We selected several circRNAs which were highly expressed and differentially regulated in Th cells. Using Northern Blot, RT-PCR and RNA FISH we validated these candidates, verified their RNase R resistance and confirmed their location in the cytoplasm. Our data suggest that circRNAs are abundantly expressed and might have a regulatory function in proinflammatory Th lymphocytes.

P.B3.03.16

Ig-like transcript 2 (ILT2) suppresses T cell function in Chronic Lymphocytic Leukemia

M. Villa-Álvarez[1,2,3], *S. Lorenzo-Herrero*[1,2,3], *A. Gonzalez-Rodriguez*[4,2,3], *A. Lopez Soto*[1,2,3], *A. Payer*[4,3], *E. Gonzalez-Garcia*[5,3], *L. Huergo-Zapico*[1,2], *S. Gonzalez*[1,2,3];
[1]University of Oviedo, Oviedo, Spain, [2]Instituto Universitario de Oncología del Principado de Asturias (IUOPA), Oviedo, Spain, [3]Instituto de Investigación Biosanitaria del Principado de Asturias (IISPA), Oviedo, Spain, [4]Hospital Universitario Central de Asturias, Oviedo, Spain, [5]Hospital de Cabueñes, Gijón, Spain.

Introduction Chronic lymphocytic leukemia (CLL) is associated with a profound dysregulation of the immune system. Loss of T cell function is frequently caused in cancer by sustained signaling of inhibitory receptors. In this work, we analyzed the role of the inhibitory receptor Ig-like transcript 2 (ILT2) in the pathogenesis of CLL. **Methods** The expression and function of ILT2 and its ligands in different lymphocyte subsets from 52 CLL patients and 20 healthy donors were evaluated in this study. **Results** ILT2 expression was markedly reduced on leukemic cells, whereas it was increased on T cells from CLL patients, particularly in patients harboring chromosome 11q deletion, which includes the ATM gene. The expression of ILT2 ligands in leukemia cells was also deeply dysregulated. ILT2 impaired the activation and proliferation of CD4 and CD8 T cells in CLL patients, but it had no effect in leukemic cells. ILT2 downregulated the production of IL-2 by CD4 T cells of CLL patients and induced the expression of cytokines that promote the survival of leukemic cells, such as IFN-, by T cells. Importantly, ILT2 blockade restored the activation, proliferation and cytokine production of T cells. **Conclusion** Our experiments indicate that ILT2 impairs T cell function in CLL. Here, we describe a novel immune inhibitory pathway that is up-regulated in CLL and delineate a new potential target to be explored in this disease. Funding: This work was supported by the Spanish grants of Fondo de Investigaciones Sanitarias (Instituto de Salud Carlos III) PI12/01280 and PI16/01485.

P.B3.03.17

The effects of redox regulated cofilin-1 on T-cell mediated immunity

Y. Xiong[1], *I. Seeland*[1], *B. Jahraus*[1], *G. Küblbeck*[2], *G. Wabniz*[1], *J. Küblbeck*[1], *Y. Samstag*[1];
[1]Institute of Immunology, Heidelberg, Germany, [2]German Cancer Research Center, Heidelberg, Germany.

Cofilin-1 is an actin-remodeling protein, which is essential for T cell development, activation and migration. Oxidation of cofilin-1 causes it to lose its affinity for actin and to translocate to the mitochondria, which leads to T cell hypo-responsiveness or even programmed cell death. This includes both oxidation of cofilin-1 at Cys residues and dephosphorylation at Ser 3.
In terms of this, we hypothesized that cofilin-1 Cys to Ala mutants may make T cells more resistant to oxidative stress. Therefore, we investigated the functionality and regulatability of different non-oxidizable Cys to Ala mutants expressed in human peripheral blood T-cells under oxidative stress conditions. We identified a double Cys to Ala mutant that showed notable resistance towards cytotoxic H_2O_2 effects. Still, this mutant differentially rescued T cells under oxidative stress conditions with regard to sustained adhesion of T cells to APCs and formation of the immunological synapse, the phosphorylation and actin-remodeling functions of cofilin-1, T cell activation and migration.
Furthermore, we have generated T cell specific knock-in mice which express these cysteine-to-alanine mutants instead of the wildtype cofilin-1. These mice will serve as tools to clarify the role of cofilin-1 redox-regulation during T cell responses *in vivo* and its potential role in different immune-related diseases (e.g. infections, tumor immunology, or chronic inflammation).

P.B3.04 T-cell regulation - Part 4

P.B3.04.01

Superior contact avidity of Treg membrane orchestrates antigen specific suppression via stripping cognate peptide-MHCII from DC surface

B. Akkaya, *M. Akkaya, Y. Oya, J. Al Souz, A. H. Holstein, O. Kamenyeva, J. Kabat, D. W. Dorward, D. D. Glass, E. M. Shevach;
NIH, Bethesda, United States.*

Tregs are professional suppressors of the immune response, yet their mechanism of action in vivo remains unclear. We compared stoichiometry of the interactions of activated 5CC7 T cells and 5CC7 iTregs with $MCC_{88\text{-}103}$ pulsed splenic DCs by electron and confocal microscopy. Image analyses revealed that Tregs displayed a distinct morphology with finger like membrane projections at the DC binding site and uropods at the rear end within three hours of co-culture. In contrast, activated T cells maintained their round morphology. Tregs occupied a greater extent of the DC surface than activated T cells consistent with a higher binding avidity. Intravital two-photon microscopy of adoptively transferred OTII iTregs and activated OTII cells demonstrated that Tregs displayed greater volume and duration of contact with $OVA_{323\text{-}339}$ pulsed DC compared to that of activated cells. Subsequent to their high avidity interactions, 5CC7 iTregs captured $MCC_{88\text{-}103}$-I-E^k complexes from DC surface reducing the amount of MCC presented on DC. Reduced antigen presentation was not due to global suppression of the DC's ability to present antigen as 5CC7 and 3A9 iTregs only captured their cognate peptide-MHCII from MCC/HEL double pulsed DC, reducing the DC presentation in an antigen specific manner. When double pulsed DCs were cultured with iTregs specific for one peptide, separated from the iTregs, the DC failed to prime T cells specific for the antigen seen by the iTreg, but primed T cells specific for the second antigen. Altogether, we propose antigen specific depletion of peptide-MHCII complexes as a new mechanism for Treg-mediated suppression.

P.B3.04.02

Immunophenotyping of suppressor and cytotoxic lymphocyte subsetsand cytotoxic mechanisms innon-small cell lung cancer

E. Cetin Aktas[1], *A. Turna*[2], *A. Engin*[3], *D. I. Cikman*[4], *G. Ayan*[5], *G. Deniz*[1];
[1]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, İstanbul, Turkey, [2]Istanbul University, Cerrahpasa Medical School, Department of Thoracic Surgery, İstanbul, Turkey, [3]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Istanbul, Turkey, [4]Istanbul University, Cerrahpasa Medical School, Department of Medical Oncology, İstanbul, Turkey, [5]Istanbul University, Cerrahpasa Medical School, Department of Internal Medicine, İstanbul, Turkey.

Lung cancer is the leading cause of cancer-related death worldwide and non-small cell lung cancer (NSCLC) is the most common form of it. The aim of this study was to assess immunological properties of circulating T lymphocyte subsets and natural killer (NK) cells.Thirty newly diagnosed patients with T1-3N0M0 NSCLC, without a history of preoperative chemotherapy and/or radiotherapy, and 46 healthy subjects were included. Lymphocyte subsets were analyzed by flow cytometry. Cytotoxic capacity of NK and $CD8^+$ T cells were evaluated by CD107a degranulation assay. Compared to healthy subjects, NK, NKT, $CD3^+HLADR^+$ and $CD8^+CD28^-$ suppressor T cells were significantly increased, while percentage of $CD8^+CD28^+$, $CD4^+$and $CD3^+$T lymphocytes were significantly decreased in patients with NSCLC (p=0.002, p=0.005, p=0.000, p=0.001, p=0.001, p=0.02 and p=0.02, respectively). Although the cytotoxic capacity of NK cells were similar between the groups, increased CD107a expression was observed in $CD8^+$T cells in stimulated and unstimulated groups (p=0.001). The rates of lymphocyte and NK subsets were not significantly different between earliest stage (T1) patients and patients with T2-3 (p>0.05).Increased $CD8^+CD28^-$ T cells might suppress antitumor immunity. Although the numbers of NK cells were increased in NSCLC group, their cytotoxic capacity was impaired. An immunological scoring system might contribute to better understanding of the prognosis of NSCLC. The role of immune cells in different stages of cancer needs to be further studied.

P.B3.04.03

AMPK pathway in regulatory T cell is critical to maintain oxidative metabolism and keep energy balance within the tumor micro environment

***J. Divoux**, R. Valion, M. Laviron, E. Ronin, S. Gregoire, B. Salomon;*
CIMI Paris, Paris, France.

Cellular metabolism is a critical factor in immune cell activity. It has been well studied in conventional T cells (T_{conv}), where mTORC1 promotes anabolism during antigenic stimulation while AMPK favors catabolism to support memory function. Regulatory T cells (T_{reg}) are essential to maintain immune tolerance. Although they have been extensively studied, little is known about their metabolic profile. We therefore developed a model where AMPK was selectively deleted in T_{reg} (Foxp3^{CRE} Prkaa1^{Lox} mice). These mice were healthy and homeostasis of T_{reg} and T_{conv} seemed unchanged. However, AMPK deficient T_{reg} showed decreased mitochondria activity and palmitate harnessing as well as increased mTORC1 activity. In a tumor context, the proportion of tumor-infiltrating T_{reg} was decreased in Foxp3^{CRE} Prkaa1^{Lox} mice which correlated with a better activation of T_{conv} and slowed tumor growth. Analysis are underway to further evaluate these metabolic changes and their impact on the stability, migration and survival of tumor-infiltrating T_{reg}.

P.B3.04.04

Chloroquine promotes up-regulation of CD39 expression and suppressive function during TGF-beta mediated Treg induction

***M. C. Gerner**, L. Ziegler, R. L. Schmidt, K. G. Schmetterer;*
Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria.

Regulatory T-cells (Treg) are a subpopulation of CD4$^+$ T-cells, associated with immunosuppression and preservation of self-tolerance. Treg can suppress activation of effector T-cells through various mechanisms including the degradation of ATP into the suppressive molecule adenosine through the ectoencymes CD39 and CD73. The mechanisms regulating CD39 on CD4$^+$ T-cells are yet unknown and is independent of FOXP3. Previous studies in tumor cells have described that inhibition of autophagy leads to the up-regulation of CD39 expression. Thus, we hypothesized a similar correlation in T-cells. Consequently, we investigated signals leading to the *de novo* induction of CD39 on iTreg and assessed the influence of chloroquine, an autophagy inhibitor, on this process. Naïve CD4$^+$CD25$^-$CD39$^-$ T-cells were activated *in vitro* with anti-CD3/CD28 coated microbeads in the presence of IL-2 and TGF-β/*all trans* retinoic acid (atRA) with or without chloroquine. First experiments revealed that TGF-β/atRA led to a stronger induction of CD39 on the CD25^{high}CD127^{low} iTreg population than culture in IL-2 alone. The combination of TGF-β/atRA + chloroquine boosted CD39- and FOXP3 expression, and led to a stronger suppressive function *in vitro* compared to TGF-b/atRA Treg-induction without chloroquine. Intriguingly, T-cells cultured in chloroquine without TGF-b/atRA did not induce iTreg. Thus, chloroquine synergizes with TGF-b/atRA signaling to enhance tolerogenic functions during iTreg induction. The exact molecular mechanisms underlying these observations will be the focus of further studies. In conclusion, our studies reveal that additional signals may influence induction of Treg by TGF-beta/atRA which may allow to develop strategies for the manipulation of T-cell tolerance.

P.B3.04.05

Role of the zinc-finger protein MAZR during iNKT cell lineage differentiation

*M. J. Orola[1,2], C. Tizian[1,3], **A. F. Gülich**[4], I. Taniuchi[5], W. Ellmeier[1], S. Sakaguchi[1];*
[1]Institute of Pathophysiology, Infectiology and Immunology, Medical University Vienna, Vienna, Austria, [2]Institute of Specific Prophylaxis and Tropical Medicine, Medical University Vienna, Vienna, Austria, [3]Charité, Universitätsmedizin Berlin, Institute of Microbiology Infection and Immunology, Germany, [4]Institute of Pathophysiology, Infectiology and Immunology; Medical University Vienna, Vienna, Austria, [5]Laboratory for Transcriptional Regulation, RIKEN Center for Integrative Medical Sciences, Vienna, Japan.

Myc-associated zinc finger-related factor (MAZR, also known as PATZ1) is an essential transcription factor for CD8$^+$ lineage differentiation of "conventional" T cells, However, its role in innate-like T cells such as invariant NKT (iNKT) cells is not known. Here we showed that T cell lineage-specific deletion of MAZR resulted in an enlargement of CD44$^+$NK1.1$^-$ stage 2 iNKT cells, accompanied with the elevated expression of CD4 and Th-inducing Pox virus and zinc finger/Krüppel-like factor (ThPOK). In the absence of MAZR there is an increase in iNKT2 cell number, concurrent with a reduced number of iNKT17 cells, and that this alteration occurred in a cell intrinsic manner. Consistent with the altered iNKT cell lineage differentiation, MAZR-deficient iNKT cells displayed an enhanced production of IL4, along with a reduction in IL17A production, both upon *in vitro* PMA/ionomycin and *in vivo* alpha-galactosylceramide (α-GalCer) stimulation. Ongoing studies address the impact of MAZR on liver injury in α-GalCer- and Concanavalin A (ConA)-induced hepatitis models. Together, our study identified MAZR as an essential factor regulating the lineage differentiation of iNKT2 and iNKT17 cell.

P.B3.04.06

Regulatory T cells promote metabolic reprogramming of CD8 T cells from cytotoxic effector to quiescent memory state

***V. Kalia**[1], A. Vegaraju[2], Y. Yuzefpolskiy[1], H. Xiao[2], S. Sarkar[1];*
[1]University of Washington School of Medicine, Seattle, United States, [2]Seattle Children's Research Institute, Seattle, United States.

Metabolism adapts to support distinct T cell functional states as naïve cells differentiate into effector and memory cells. TCR, costimulatory and cytokine signals rapidly switch the metabolic program of quiescent naïve cells from oxidation of fatty acids and glucose via OXPHOS to a program of anabolism via augmented aerobic glycolysis to support the proliferative burst and cytotoxic function. After antigen clearance, conversion from cytotoxic effector state to quiescent memory state is associated with a metabolic switch to catabolic processes such as fatty acid oxidation and autophagy. Availability of antigen, cytokine and nutrients is believed to largely orchestrate metabolic transitions of naïve, effector and memory CD8 T cells. Nonetheless, it remains unclear how anatomic micro niches and interactions with other immune cells (such as CD4 T cell help or regulation by Treg cells) impact CD8 T cell differentiation-associated metabolic remodeling. Here we show that anatomic location of memory-fated effector CD8 T cells within the splenic micro niches dictates their metabolic state and protective functional outcome. Our studies further reveal a critical requirement of Treg cells in promoting metabolic remodeling of memory-fated effector CD8 T cells as they enter quiescence after antigen clearance. Ablation of Treg cells led to specific metabolic dysregulation in the memory-fated CD8 T cell subset, resulting in compromised memory function. These studies define novel molecular targets that may be exploited to manipulate metabolism, migration and memory function during vaccination.

P.B3.04.07

The role of granzyme B in the function of regulatory T cells induced by B cells

***S. Kang**, S. Lin, B. Chiang;*
Graduate Institute of Immunology, Collage of Medicine, Taipei, Taiwan.

Introduction. The regulatory T cells have been found to be essential for immune modulation and maintenance of tolerance. Recent studies demonstrated a novel subset of regulatory T cells, the Treg-of-B cells, which are induced by B cells. Our group is studying the characteristics and functions of Treg-of-B cells, and their implications on autoimmune and allergic diseases.
Materials and Methods. In this study, we co-cultured naïve T and B cells and isolated the B-cell-induced Treg-of-B cells after activation. We examined and characterized the cells with qPCR, ELISA and flow cytometry, and also studied their immune suppression ability. In addition, we also like to investigate the role of granzyme in the function of Treg-of-B cells.
Results. The results showed that Treg-of-B cells were a subset of CD4$^+$CD25$^+$ regulatory cells with significant immune suppressive function. This subset of T cells did not express the key markers of naturally occurring regulatory T cells, Foxp3, and secreted higher level of granzyme B. We will like to further study the role of granzyme B in the function of Treg-of-B cells with granzyme B knockout mice.
Conclusion. Here we demonstrated a novel subpopulation of regulatory T cells induced by co-culture with B cells. This particular subpopulation of Treg-of-B cells express LAG-3, CTLA-4 and produced IL-10, however, they do not express Foxp-3 gene. We believe that granzyme B might also play a critical role in the function of Treg-of-B cells.

P.B3.04.08

Tracing of the NKT1 differentiation pathway using Hobit reporter mice

***N. A. M. Kragten**[1], L. Parga Vidal[1], F. M. Behr[1,2], R. L. Taggenbrock[1], T. H. Wesselink[1], R. Stark[1], K. P. van Gisbergen[1,2];*
[1]Dept. of Hematopoiesis, Sanquin Research and Landsteiner Laboratory AMC/UvA, Amsterdam, Netherlands, [2]Dept. of Experimental Immunology, AMC, Amsterdam, Netherlands.

Natural killer T (NKT cells) are unconventional T cells that play a regulatory role in a broad range of infectious diseases and autoimmune disorders. We have previously shown that the transcription factor Homologue of Blimp-1 in T cells (Hobit) is highly upregulated in NKT1 cells and plays an essential role in the development of the NKT1 lineage, but not in the NKT2 and NKT17 lineages.
To address the differentiation pathway of NKT1 cells, we have developed a reporter mouse that expresses the fluorescent protein tdTomato, Cre recombinase and the diphtheria toxin receptor (DT) under the control of the Hobit promoter. Using the tdTomato reporter as a read-out for Hobit expression, we found that mature NKT1 in contrast to immature NKT cells, NKT2 cells and NKT17 cells expressed Hobit. To examine the potential of thymic precursors to generate NKT1 cells, we depleted tdTomato+ NKT1 cells using DT and analyzed the re-appearance of these cells. We found that mature NKT1 cells did not re-develop after depletion, suggesting that the population is stably maintained in the periphery independent of thymic input. Using lineage tracer mice (Hobit CRE x Rosa26-(floxed STOP)-YFP mice), we addressed the potential of NKT1 to contribute to the development of the other NKT lineages. We did not find YFP expression in NKT2 and NKT17 cells, suggesting that Hobit+ NKT1 cells lack NKT2 and NKT17 potential. Thus, our data show that NKT1 form a thymus-independent population of terminally differentiated NKT cells in the peripheral organs.

P.B3.04.09

Human CD8+HLA-DR+ regulatory T cells share phenotypic and functional features with classical CD4+Foxp3+ regulatory T cells

***A. Machicote**, S. Belén, P. Baz, L. A. Billordo, L. Fainboim;*
Laboratorio de Inmunogenética, Instituto de Inmunología, Genética y Metabolismo, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires-Consejo Nacional de Investigaciones Científicas y Técnicas, CABA, Argentina.

Introduction: We previously described a human $CD8^+$ regulatory T cell subset, constitutively expressing the HLA-DR molecule ($CD8^+HLA\text{-}DR^+$ Tregs), which suppresses the proliferation of autologous PBMCs through cellular contact and CTLA-4. We aimed to characterize the regulatory signature of these Tregs, to identify additional mediators involved in the regulatory mechanism, and to evaluate their exhaustion status.
Materials and Methods: Peripheral and cord blood samples were obtained from healthy donors. Phenotypic markers were analyzed by flow cytometry. Suppression assays were performed with CellTrace Violet-stained PBMCs as responders, co-cultured with sorted $CD8^+HLA\text{-}DR^+$ or $CD8^+HLA\text{-}DR^-$ cells, and activated with aCD3/aCD28. Cytokine secretion was analyzed after PMA/Ionomycin stimulation.
Results: In comparison with $CD8^+HLA\text{-}DR^-$ cells, $CD8^+HLA\text{-}DR^+$ Tregs showed an increased frequency of PD-1 ($p<0.0001$) and TIGIT ($p<0.0001$), and lower expression of CD127 ($p=0.0009$). Consistent with the high expression of PD-1, the addition of PD-1 and PD-L1 neutralizing antibodies abrogated the suppression effect of $CD8^+HLA\text{-}DR^+$ Tregs ($p=0.0015$), acting preferentially on $CD8^+$ responder cells. In comparison with $CD8^+HLA\text{-}DR^-$ cells, $CD8^+HLA\text{-}DR^+$ Tregs presented a higher frequency of IFNg ($p=0.0098$), TNFa ($p=0.0004$) and CD107a ($p=0.0006$) after PBMCs stimulation, and a higher proliferation rate ($p=0.0037$). Within adult PBMCs, 90±2% of $CD8^+HLA\text{-}DR^+$ have a differentiated- or memory-like phenotype, that contrasted with cord blood $CD8^+HLA\text{-}DR^+$ Tregs being mostly naïve (86±6%) ($p<0.0001$).
Conclusions: $CD8^+HLA\text{-}DR^+$ Tregs show high similarities with classical $CD4^+Foxp3^+$ Tregs, including expression of PD-1, TIGIT and CD127 molecules, and abrogation of suppressor capacity by anti-PD-1 antibody. Our results also confirmed that $CD8^+HLA\text{-}DR^+$ Tregs are not exhausted cells.
Grant Nº: ANPCYT PICT2014 0925

P.B3.04.10

Heterogeneity of one-cell derived mesenchymal stem cells on immunomodulation of lymphocyte subsets

***P. Martinez-Peinado**[1], S. Pascual-García[1], A. López-Jaen[1], F. Navarro-Blasco[1], E. Roche[2], J. Sempere-Ortells[1];*
[1]University of Alicante, San Vicente del Raspeig, Spain, [2]University Miguel Hernández, Elche, Spain.

Mesenchymal stem cells (MSC) are widely used in cell therapy to treat autoimmune diseases because of their immunomodulatory properties. However, the results of such clinical trials are, in many cases, incongruent. The heterogeneous character of MSC may be responsible for these results. In this study, we analysed the differences of five MSC clones to exert their *ex vivo* immunomodulation.
We conducted proliferation assays of CD3, CD4 and CD8 purified lymphocytes co-cultured with the different MSC clones, as well as assays to analyse the effect of these clones on the Treg population in co-cultures with PBMC. We also analysed the effect of these clones on the viability of PBMC in the presence of a pro-apoptotic stimulus. Finally, Th1/Th2 cytokine environment of co-cultures with PBMC was characterised as well. We also checked the effect of conditioned media on the above parameters.
The clones inhibited the proliferation of $CD3^+$ and $CD4^+$ lymphocytes with different intensity. Surprisingly, all the clones promoted the proliferation of $CD8^+$ lymphocytes, with significant differences between them. MSC clones and their CM increased the number of Treg with varying intensities. Clones promoted both increases and decreases in the viability of UV-treated PBMCs. Finally, the clones also promoted heterogeneous cytokine environments.
Considering all these results, it seems that different clones can promote a wide range of responses, from anti-inflammatory to pro-inflammatory. This may be important in the standardization of the design of personalized cell therapy protocols, thus decreasing the undesirable outcomes that currently exist in this type of therapy.

P.B3.04.11

The AMP analog AICAR modulates the Treg/Th17 axis through AMPK-mediated enhancement of fatty acid oxidation

***K. A. Mayer**[1], G. A. Gualdoni[1,2], L. Göschl[1,2], N. Boucheron[1], W. Ellmeier[1], G. J. Zlabinger[1];*
[1]Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria, [2]Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria.

Introduction: T cells must tightly regulate their metabolic processes to cope with varying bioenergetic demands depending on their state of differentiation. The metabolic sensor AMPK is activated in states of low energy supply and modulates cellular metabolism toward a catabolic state. Although this enzyme is known to be particularly active in regulatory T (Treg) cells, its impact on T helper (Th) cell differentiation is poorly understood. Results: We investigated the impact of several AMPK activators on Treg differentiation and found that the direct activator AICAR (5-aminoimidazole-4-carboxamide ribonucleotide), but not the indirect activators metformin and 2-deoxyglucose, strongly enhanced Treg cell induction. Further investigation of the metabolic background of our observations revealed that AICAR enhanced both cellular mitochondrogenesis and fatty acid uptake. Consistently, increased Treg-induction was entirely reversible upon inhibition of fatty acid oxidation. Translating our findings to an in vivo model, we found that the substance enhanced Treg cell generation upon IL-2 complex-induced immune stimulation. Strikingly, AICAR had no effect in AMPK-deficient T cells, therefore highlighting the important role of AMPK in Treg expansion. Discussion: We provide a previously unrecognized insight into the delicate interplay between immune cell function and metabolism and delineate a potential novel strategy for metabolism-targeting immunotherapy.

P.B3.04.12

Cross talk between naïve CD4+ T cells and cancer and normal adipose derived mesenchymal stem cells (ASCs) and induction of distinct subsets of regulatory T cells

***M. Razmkhah**[1], M. Fakhimi[1], A. Talei[2], M. Habibagahi[3], A. Ghaderi[4];*
[1]Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of, [2]Breast Diseases Research Center (BDRC), Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of, [3]Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of, [4]Shiraz Institute for Cancer Research, Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, Islamic Republic of.

Tumors are complex tissues containing many different cell types, including stromal cells, cells of the immune system and mesenchymal stem cells (MSCs). MSCs play a significant role as immunomodulators in the tumor microenvironment through producing different cytokines. The present study aimed to clarify Treg subsets and their cytokines after exposing naïve T lymphocytes to adipose derived mesenchymal stem cells (ASCs). Human ASCs were obtained and characterized from breast adipose tissues of a breast cancer patient and a normal individual. Naïve CD4+T cells were purified from peripheral blood of healthy donors using magnetic cell sorting (MACS) and were cultured for 5 days with cancer and normal ASCs. The phenotype of harvested regulatory T cells and the production of IL-10, TGF-β and IL-17 by T cells were evaluated using flow cytometry and ELISPOT assay, respectively. As a result, CD4+CD25+Foxp3+CD45RA+ and CD4+CD25+Foxp3+Helios+ Treg cells increased significantly in the presence of normal-ASCs and breast cancer-ASCs compared to those cultured without ASCs (P values= 0.002 and 0.005). Coculturing of naïve T cells with cancer-ASCs resulted in significant expansion of CD4+CD25-Foxp3+Helios+ T cells. Also, the percentage of CD25+Foxp3+CD73+CD39+ T cells was significantly upregulated in the presence of cancer-ASCs (P value=0.005). The production of IL-10, TGF-β and IL-17 by T cells was increased with both normal- and cancer-ASCs but cancer-ASCs had more significant effects on IL-10 and TGF-β production (P value< 0.05). Results of the present study suggest more evidences for the immunosuppression of ASCs on T lymphocytes and deviation of these cells to distinct regulatory phenotypes providing conditions in favor of immune evasion and tumor growth.

P.B3.04.13

A role for RORα-expressing CD4 cells in the immune response to the helminth *Nippostrongylus brasiliensis*

***J. Roberts**, C. Schwartz, P. Fallon, E. Hams;*
School of Medicine, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland.

The transcription factor Retinoic acid-related Orphan Receptor (ROR)-α is a member of the ROR family, also including RORβ and RORγ. RORα has roles in cerebellar development, circadian rhythm and inflammatory responses and is required for the development of Th17 cells and group 2 innate lymphoid cells (ILC2). Infection of mice with the helminth *Nippostrongylus brasiliensis* induces a strong type 2 response, characterized by localised recruitment of eosinophils, alternatively activated macrophages and Th2 cells, which is required to clear the worms from the host. It has previously been shown that ILC2s are crucial for *N. brasiliensis* clearance, herein we sought to investigate the potential role of other RORα-expressing cells. Infecting staggerer mice ($Rora^{sg/sg}$), which have a natural mutation in the gene encoding Rora resulting in a premature stop codon, with *N. brasiliensis* we show a delay in worm rejection compared to wild type mice. Furthermore, we have observed an altered type 2 immune response in helminth infected $Rora^{sg/sg}$ mice, associated with decreased eosinophil and Th2 cell recruitment. Using the Cre-lox system we have generated mice with specific deletion of Rora in CD4+ T cells ($Rora^{fl/fl}$CD4Cre) and ILC2 ($Rora^{fl/sg}$IL-7RaCre/+). Infection of these mice has shown reduced worm burden in the absence of Rora-expressing CD4+ T Cells, compared to a delay in worm clearance in the absence of ILC2s. These data suggest a role for Rora-expressing CD4+ T cells in the progression of a functional type 2 response to infection with the helminth *N. brasiliensis*.

P.B3.04.14

The role of anergy-related molecules in development and function of regulatory T cells induced by B cells

***T. Yeh**, B. Chiang;*
Graduate Institute of Immunology, Taipei, Taiwan.

Introduction: Naïve B cell could act as antigen presenting cell to convert $CD4^+CD25^-$ T cell into $FOXP3^-$ regulatory T cell which called Treg-of-B cells. Treg-of-B cell suppressed effector T cell proliferation by cell-cell contact manner which is different from other Treg population, included tTreg, Tr1 and Th3. In seraval studies of autoimmune disease animal models, Treg-of-B cell could alleviated inflammation. Anergy is a mechanism of peripheral tolerance which caused by weak or non-costimulatory signal. Treg are considered to be naturally anergic because of its hypoproliferative characteristics and low IL-2 production with involvement of anergy associated factors. Treg-of-B cells have been found also with the anergic phenotype. Materials and Methods: In this study, we aim to identify the anergic phenotype of Treg-of-B cells, and analyze the anergy associated genes expression. We established several induction systems to discuss how antigen specificity and co-stimulatory signal affect Treg-of-B cell development and function. Then we will further analyze the IL-2 suppressor - Aiolos expression of Treg-of-B cell. Results: Treg-of-B cell exerted anergic phenotype but did not express NFAT associated anergic factors. Treg-of-B cell Induced with antigen specific B cell or higher co-stimulatory signal slightly restored the suppressive function and accompanied with decreased Aiolos expression. Conclusions: Antigen specificity and high costimulatory signal caused Treg-of-B cell to be more activated which affect their suppressive function. Different expression of Aiolos suggested that it might be associated to the anergic status of Tre-of-B cell. but whether Aiolos involved in development and function of Treg-of B cell still needs clarification.

P.B3.04.15

Characterization of the FOXP3-regulated anti-proliferative gene signature in thymus-derived regulatory T-cells

***L. Ziegler**, M. Gerner, R. Schmidt, K. Schmetterer;*
Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria.

Background: The transcription factor Forkhead-box protein 3 (FOXP3) is master regulatory factor for thymic-derived regulatory T-cells (tTreg) and controls the expression of several hundred genes. Still our knowledge about this FOXP3-regulated gene signature is far from complete. Using microarray screenings of FOXP3-transgenic human T-cells, we identified several genes, which are regulated by FOXP3 and have been described as cell-cycle regulators in various cell types: Juxtaposed with another zinc finger 1 (JAZF1), MAX-interacting protein 1 (MXI1), B-cell translocation gene 1 (BTG1) and Glucocorticoid-induced leucine zipper (GILZ). Methods: Expression of these genes was measured by RT-PCR in resting or anti-CD3/anti-CD28 activated FOXP3- or control-vector transduced T-cells or CD4+CD25+ Treg. cDNAs for the genes were cloned into a retroviral expression vector and the effects of overexpression in CD4+ T-cells on proliferation, marker expression and cytokine secretion were measured. Results: Expression of all four genes was induced in FOXP3-transgenic T-cells and tTreg following activation. In contrast, expression in control-vector transduced or CD4+ effector T-cells was significantly down-regulated. Overexpression of GILZ showed a marked up-regulation of the tTreg markers CD25 and GITR. Following activation, proliferation of all transgenic T-cells was significantly decreased. All four genes showed effects on the secretion of different cytokines, including IL-2, IL-17 and IFN-γ. Conclusion: JAZF1, MXI1, BTG1 and GILZ are involved in the maintenance of hypo-responsiveness in Treg and contribute to their reduced cytokine secretion. Thus, our data add to the understanding of the FOXP3-regulated gene signature in Treg and may open new strategies for the manipulation of these cells.

P.B3.04.16

Platelets induce a regulatory phenotype in T cells via the expression of GARP

***N. Zimmer**[1], S. A. Hahn[1], S. Wilden[1], E. Walter[2], K. Jurk[2], S. Grabbe[1], A. Tuettenberg[1];*
[1]Department of Dermatology, Mainz, Germany, [2]Center for Thrombosis and Hemostasis Mainz (CTH), Mainz, Germany.

Introduction: Beside their main function in hemostasis, platelets are important modulators of the innate and adaptive immunity through their interaction with immune cells. Glycoprotein A repetitions predominant (GARP), first described on platelets and as an activation marker on regulatory T cells (Treg), is known to modulate the bioavailability of TGF-beta and is therefore involved in the regulation of the peripheral immune responses. Recently, we were able to show that the soluble form of GARP (sGARP) has strong regulatory and anti-inflammatory properties in vitro and in vivo. sGARP leads to induction of peripheral Treg (pTreg) as well as to inhibition of tumor antigen-specific $CD8^+$ T cells.
Materials and Methods: In the present study, we investigated the effect of platelets on the differentiation and phenotype of $CD4^+$ T cells dependent on GARP.
Results: sGARP was detected in the supernatant of activated platelets. Consequenlty, platelets were able to inhibit dose dependently the proliferation and cytokine production, while inducing a strong Foxp3 expression and a suppressive capacity in coculture. Using a blocking anti-GARP mAb, we were able to reverse these effects.
Conclusion: Our data give evidence that platelets are capable to induce pTreg in a GARP-dependent manner. The expression and shedding of GARP by platelets could be of importance in diseases like cancer, where poor prognosis and metastasis are associated with elevated numbers of circulating platelets (thrombocytosis).
This work is financially supported by the Wilhelm Sander-Stiftung.

P.B3.04.17

Tr1-like ($IL\text{-}10^+$, $IFNg^-$, $IL4^-$) cells in peripheral blood are part of the T effector memory pool and are preferentially stimulated via CD55

***I. A. Charles**, J. M. Ramage, I. Spendlove, Cancer Immunotherapy group;*
The University of Nottingham, Nottingham, United Kingdom.

Effector T cells arise from naïve T cell stimulation and enter a long term memory pool. While they can adapt their function to specific environmental cues, sometimes called plasticity, their phenotype remains broadly fixed. Unlike natural T regulatory cells that emerge from the thymus with a defined phenotype ($CD4^+CD25^{hi}CD127^{lo}FoxP3^+$), there is uncertainty over the characterisation of inducible Tr1-like regulatory cells. In this study we stimulated human total $CD4^+$ T cells with PMA/ Ionomycin. A Small (<5%) population were $IL\text{-}10^+$ $IL\text{-}4^-$, IFN-γ^- and expressed other markers commonly associated with Tr1-like cells. These included; CD49b, LAG-3, CD226, PD-1, CTLA-4, and TIM-3. However they were negative for FoxP3 expression. Furthermore, we show that these Tr1-like cells form part of the immunological memory and reside predominantly within the effector memory ($CD62L^-$ $CD45RO^+$, TEM) pool. Unlike the majority of other studies where Tr1 is generated by chronic stimulation of PBMCs in the presence of IL-10 for several days, as far as we are aware, this is the first report characterising Tr1 directly ex vivo from human peripheral blood. We have also demonstrated that these cells respond specifically to costimulation via CD97-CD55 to drive proliferation and maintain the IL-10 single positive, Tr1-like phenotype outlined above. This supports the idea that once differentiated from naïve precursors, Tr1-like cells respond to CD55 costimulation to maintain a small (<5%) pool with a committed $IL\text{-}10^+$ $IL\text{-}4^-$IFN-γ^- phenotype.

P.B3.04.18

Co-stimulatory activity of TLR7/8 and STING ligands on interferon-γ induction in human γδ T cells

***R. D. Serrano**[1], B. Putschli[2], C. Coch[2], G. Hartmann[2], D. Wesch[1], D. Kabelitz[1];*
[1]Institute for Immubnology/University of Kiel, Kiel, Germany, [2]Institute of Clinical Chemistry and Clinical Pharmacology, Bonn, Germany.

Human phosphoantigen-reactive Vγ9Vδ2 γδ T cells are potent producers of pro-inflammatory cytokines including TNF-α and IFN-γ. An expression of several Toll-like receptors (TLRs) on/in human γδ T cells inducing direct co-stimulatory effects after ligation with corresponding ligands has been reported, while strong co-stimulation can also provided by TLR activated monocytes. In our study, we have analyzed the IFN-γ induction in peripheral blood γδ T cells in response to phosphoantigen (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) in the absence or presence of a variety of ligands for TLR7, TLR8 and stimulator of interferon genes (STING). Induction of IFN- γ expression in Vγ9-expressing γδ T cells was measured by intracellular flow cytometry after 6 to 48 hours either within PBMC or in cocultures of purified γδ T cells and purified monocytes. In the absence of HMBPP stimulation, some IFN-γ expression was induced by TLR8 (TLR8-506 and ss40 RNA) and TLR7/8 ligands (Resiquimod and 9.2s RNA). In the presence of low concentrations of HMBPP, the same ligands strongly co-stimulated IFN-γ expression in γδ T cells. In addition, adenosine- but not guanosine/inosine-containing cyclic dinucleotide STING ligands also exerted strong co-stimulatory activity only in company with HMBPP. The same ligands, however, inhibited the proliferative expansion of freshly isolated γδ T cells cocultured with monocytes in response to HMBPP and zoledronic acid. Microscopic inspection suggested that the adenosine-containing cyclic dinucleotides induced cell death in purified monocytes, in line with a recent reports. Our data indicate a dual role of some PRR ligands in human γδ T cell activation, a notion which requires further studies to uncover the underlying mechanisms.

P.B4.01 T-cell activation and exhaustion - Part 1

P.B4.01.01
Switching cytotoxicity to inflammation pattern in relation to cutaneous melanoma stages

*M. Surcel[1], **C. Constantin**[1,2], R. Huica[1], A. Munteanu[1], I. Pirvu[1], G. Isvoranu[1], O. Bratu[3], D. Ciotaru[1], C. Ursaciuc[1], M. Neagu[1,4,2];*
[1]"Victor Babes" National Institute of Pathology, Bucharest, Romania, [2]Colentina University Hospital, Bucharest, Romania, [3]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, [4]Faculty of Biology, University of Bucharest, Bucharest, Romania.

Cutaneous melanoma (CM) represents only 4% of all skin cancers, but it is responsible for 80% of skin cancer deaths, thus identifying new prognostic and evolution markers remains a constant endeavor. **Methods.** Using flow-cytometry peripheral blood form CM patients diagnosed in all the stages was quantified for total T cells ($CD3^+$), B cells ($CD3^-CD19^+$), NK cells ($CD3^-CD16^+$ and/or $CD56^+$), T helper cells ($CD3^+CD4^+$), T supressor/cytotoxic cells ($CD3^+CD8^+$) and regulatory T cells ($CD4^+/CD25^+/FOXP3^+$) (T-reg); % of lymphocytes secreting IL-2, IFN-γ and TNF-α. **Results.** The main changes found in 83% of patients were the decrease of both percentage and absolute number of T-CD8+ with an increase of $T\text{-}CD4^+/T\text{-}CD8^+$ ratio. T-reg values were found elevated in 53% of cases; 63% of patients with increased T-reg presented decreased percentage of T-CD8+ lymphocytes.
Significant changes in the % of lymphocytes synthetizing IFN-γ (17±7% patients compared to 9±3% in controls, $p<0.02$) and in TNF-α synthesis (33±9% patients, compared to controls 22±11%, $p<0.05$) were found. **Conclusions.** CM cellular profile suggests a trend of dissociation of antitumor immune response efficiency. Firstly, the decreasing of T-cytotoxic cells and the increasing of regulatory T cells diminish the cytotoxic activity. The increasing of secretory cells % for pro-inflammatory cytokines (IFN-γ and TNF-α) may be relative, being the consequence of increasing the percentage of T-helper cells, indicating the switch of antitumour activity from cytotoxicity to inflammation. **Acknowledgement:** This work was supported by Core Program, implemented with the support NASR, project no. 18.21.02.02 and PN-III-P1-1.2-PCCDI-2017-0341/2018.

P.B4.01.02
Effective expansion and reprogramming of tumor infiltrating lymphocytes from non-small cell lung cancers

***R. De Groot**[1], M. M. van Loenen[1], A. Guislain[1], M. M. Van den Heuvel[2], J. De Jong[2], R. M. Spaapen[1], D. Amsen[1], J. B. Haanen[2], K. Monkhorst[2], K. J. Hartemink[2], M. C. Wolkers[1];*
[1]Sanquin Research, Amsterdam, Netherlands, [2]Netherlands Cancer Institute, Amsterdam, Netherlands.

Non-small cell lung cancer (NSCLC), the second most occurring type of cancer, is highly recurrent with limited 5-year survival. NSCLCs contain high numbers of T cells, and although suggestive of tumor reactivity, these tumor infiltrating lymphocytes (TILs) are unable to eradicate the cancer. Here we investigated whether TILs from NSCLC can be reprogrammed and used for autologous T cell therapy. TILs were isolated from tumor tissues and reprogrammed via in vitro activation using a standard TIL expansion protocol. Strikingly, the vast majority of expanded TILs from primary NSCLC tumor tissues (stage I-IIIA) were tumor reactive; TILs from 13/17 donors (76,5%) produced inflammatory cytokines in response to tumor digests, but not in response to distal non-tumorous lung tissues. Tumor responses ranged from 1%-35% of cytokine-producing TILs, which correlated with expression of activation markers CD137 on tumor-reactive T cells and CD40L on tumor-reactive $CD4^+$T cells. Importantly, TIL cultures with high cytokine responses contained polyfunctional T cells that produced IFN-γ together with TNF-α and/or IL-2. Strikingly, high expression of $CD103^+/CD69^+$on $CD8^+$T cell, and the presence of B cell as well as regulatory T cell infiltrates in the tumors were strong predictors of the intensity of tumor reactivity in expanded TILs. Our data show that reprogramming of TILs from NSCLC is effective, strongly suggesting that autologous T cell therapy should be considered to treat NSCLC patients.

P.B4.01.03
The SPPL3-controlled tumor glycosphingolipid repertoire determines MHC class I functionality

***A. A. de Waard**[1], M. L. Jongsma[1,2], M. Raaben[3], T. Zhang[2], V. A. Blomen[3], R. Platzer[4], S. Bliss[1], S. Holst[2], P. Schatzlmaier[4], L. J. Janssen[2], A. Mulder[2], M. H. Heemskerk[2], F. H. Claas[2], M. Griffioen[2], H. S. Overkleeft[5], J. B. Huppa[4], M. Wuhrer[2], T. R. Brummelkamp[3], J. Neefjes[2], R. M. Spaapen[1];*
[1]Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands, [2]Leiden University Medical Center, Leiden, Netherlands, [3]Netherlands Cancer Institute, Amsterdam, Netherlands, [4]Institute for Hygiene and Applied Immunology, Vienna, Austria, [5]Leiden University, Leiden, Netherlands.

MHC class I (MHC-I) molecules are key regulators of T cell activation. Processes catalyzing functional MHC-I antigen presentation are therefore often dysregulated in cancer. To identify such processes, we performed a state-of-the-art genome-wide screen using haploid genetics. We identified the intramembrane protease SPPL3 as a novel positive regulator of antigen presentation as validated by a reduction of CD8+ T cell responses against CRISPR/Cas9 engineered SPPL3 knockout (KO) cells.
Additional genome-wide screens revealed that SPPL3 controls enzymes of the glycosphingolipid (GSL) synthesis. Indeed, mass spectrometry of the GSLs showed that the repertoire is completely altered in SPPL3 KO cells. Moreover, systematically knocking out enzymes in the GSL synthesis pathway enabled us to create a map of the GSL repertoire affecting MHC-I. In combination with the mass spectrometry data, we discovered that lacto- and neolactoseries GSLs generated by the enzyme B3GNT5, hinder antibody and receptor association with MHC-I. We determined that SPPL3 controls this process by proteolytically inactivating B3GNT5 and therefore (neo-)lactoseries GSLs are only present in the absence of SPPL3. Screening a panel of diverse tumor cell lines revealed that GSLs impair MHC-I in AML and several other types of tumors, suggesting that GSLs may be involved in escape from T cell surveillance.
In conclusion, GSL regulation by SPPL3 is a novel regulatory mechanism of functional MHC-I antigen presentation. Targeting GSL synthesis may therefore constitute a novel mode of immunotherapy for cancer.

P.B4.01.04
Tumor infiltrating T cells: complete workflows allow faster and improved flow cytometric analysis of syngeneic mouse tumors

***C. Evaristo**, R. Siemer, D. Agorku, J. Brauner, O. Hardt, C. Dose, A. Richter;*
Miltenyi Biotec, Bergisch Gladbach, Germany.

Immunotherapies have proven clinical efficacy in multiple cancers. Syngeneic mouse models represent the gold standard to develop and analyze effects of immunotherapy. However, phenotypic and functional analysis of tumor-infiltrating leukocytes (TILs) is technically challenging and time/labor intensive. Importantly, T cells are embedded in a highly immunomodulatory microenvironment, hindering unbiased functional characterization. It is fundamental to use innovative tools to streamline workflows and generate reliable data.
We established complete workflows combining tissue-storage, dissociation, T cell-isolation and phenotyping. Tissues were processed immediately or stored in a solution that maintains cell viability and phenotype up to 48h (Tissue Storage Solution™). Tumor-dissociation was automated and optimized for epitope preservation using a tissue dissociator (gentleMACS™ Octo). Phenotypic analysis revealed that optimal enzymatic dissociation was essential for analysis of critical tumor-specific sub-populations, such as $PD1^{hi}Tim3^+Lag3^+CD39^+CD8^+$ T cells. We developed new reagents which enrich for rare tumor infiltrating T cells up to 500-fold, while maintaining activation status and phenotype. Importantly, use of recombinant REAfinity™ antibodies significantly diminished non-specific labeling of cells present in the tumor microenvironment. Finally, flow cytometric analysis was performed using an automated analyzer (MACSQuant X™). This instrument decreased total and hands-on acquisition time by facilitating fast and automated sample processing, including sample mixing and cell counting.
In conclusion, we optimized workflows including standardized processing of tumor samples, new tools for TIL isolation and automated flow cytometry. These workflows reduce experimental time and allow the performance of more complex experiments. Use of these tools can significantly increase the data quality in immuno-oncology and immunotherapy research.

P.B4.01.05
Immune profiling of the tumour microenvironment in cancer to determine patient responses to immunotherapy

***A. L. Ferguson**[1,2], J. Toh[3,2], K. Lo[4], A. Hong[2,5,6], G. Long[2,6,5], R. Scolyer[2,6,5], U. Palendira[1,2];*
[1]Centenary Institute, Sydney, NSW, Australia, [2]Sydney Medical School, The University of Sydney, Sydney, NSW, Australia, [3]Westmead Hospital, Sydney, NSW, Australia, [4]Department of Anatomical and Cellular Pathology at the Chinese University of Hong Kong, Hong Kong, Hong Kong, [5]Royal Prince Alfred Hospital, Sydney, NSW, Australia, [6]Melanoma Institute Australia, The University of Sydney, North Sydney, NSW, Australia.

Cancer immunotherapy targeting immune checkpoints and inhibitory molecules is a promising treatment option for many types of cancer. However, durable response are seen in less than 40% of patients and many experience immune-related adverse events such as the development auto-immune disease. Recent advances in tumour immunology research have found that certain immune cell subsets such as CD103+ tumour-resident CD8+ T cells are associated with improved survival in some cancers. It has become clear that the more we understand about the immune profile of the tumour micro-environment (TME) the more we will be able to predict a patients response and therefore direct the choice of immune-therapy in the clinic. In order to profile the resident immune cells and presence of immunotherapy targets in the human TME, we employed a technique that could evaluate this utilising both high-dimensional mass cytometry and spatial analysis, imaging mass cytometry (IMC). Using formalin fixed paraffin embedded human tumour tissues we designed an IMC panel to enable high-throughput immune profiling of prospectively collected tumours. This study provides novel information about the immune profile of the TME in multiple cancers. These findings may be crucial in determining better strategies for treatment of cancer by immunotherapies.

P.B4.01.06

Intratumoral T cell exhaustion in a murine pancreatic cancer model: Influence of induced regulatory T cells

A. Hanlon[1], *V. Lutz*[1], *E. Landmann*[1], *M. Huber*[2], *M. Buchholz*[1], *T. Gress*[1], *C. Bauer*[1];
[1]*Division of Gastroenterology, Endocrinology, Infectiology and Metabolism, University Hospital Giessen and Marburg, Philipps University Marburg, Marburg, Germany,* [2]*Institute for Medical Microbiology and Hygiene, Philipps University Marburg, Marburg, Germany.*

Introduction: Pancreatic cancer is one of the most aggressive malignancies with poor prognosis where new therapeutic approaches are needed. T cell exhaustion plays a critical role in at least some subtypes of pancreatic carcinoma. This $CD8^+$ cytotoxic T cell (CTL) dysfunction has evolved as a paradigm to understand tumor escape from immune surveillance. We have set up a model system of adoptive T cell transfer in order to elucidate mechanisms of T cellular exhaustion and of ways to disinhibit intratumoral T cells.
Methods: We work with the OVA model antigen system. Antigen-specific CTL were generated from transgenic OT-I mice and transferred into tumor-bearing mice. To investigate the role of induced regulatory T cells (iTreg) on our CTL, antigen-specific iTreg were co-transferred. Beside therapeutic response, ex vivo parameters were characterized, such as expression of coinhibitory receptors (PD-1, TIM-3, LAG-3, CTLA-4), Lamp-1-associated degranulation and intracellular production of IFN-γ and TNF.
Results: We have established growth chracteristics of PancOVA and intratumoral infiltration kinetics of effector CTL. Additionally, in vitro and in vivo characterization of iTreg behavior was performed and also their effect on cytotoxic T cell responses investigated. The induction of T cellular dysfunction was exposed as an active, antigen- and contact-dependent process.
Conclusions: We intend to validate our results in an orthotopic tumor model to approximate to real cancer conditions. Furthermore, mechanisms of iTreg-induced T cell dysfunction will be investigated using FOXP3-IRES-mRFP (FIR) OT-II mice in an in vivo/in situ approach using a dorsal skinfold chamber model to visualize intratumoral T cells.

P.B4.01.07

$CXCR5^+PD\text{-}1^+$ $CD8^+$ T-cells in human blood and lymph nodes

T. Hofland[1], *E. B. Remmerswaal*[1], *F. Bemelman*[1], *M. Levin*[2], *A. P. Kater*[1,3], *S. H. Tonino*[1,3];
[1]*AMC Amsterdam, Amsterdam, Netherlands,* [2]*Albert Schweitzer Hospital, Dordrecht, Netherlands,* [3]*LYMMCARE, Amsterdam, Netherlands.*

Re-invigorating exhausted T-cells with immune checkpoint blockade (ICB) has shown promising efficacy in the treatment of several malignancies. However, response rates vary between patients, and it's unclear which parameters predict clinical response to ICB. Recently, a subset of $CXCR5^+PD\text{-}1^+CD8^+$ T-cells has been identified in mice. This memory-like population appears to be crucial for the essential expansion of CD8+ T-cells during ICB, and is characterized by the expression of transcription factor Tcf1. Although $CXCR5^+$ $CD8^+$ T-cells have been identified in humans, it's unknown whether these cells have a similar phenotype and function as described in mice, and play a similar important role during ICB. To investigate $CXCR5^+$ $CD8^+$ T-cells, we analyzed human peripheral blood and lymph node samples from healthy donors with flow cytometry. We found $CXCR5^+PD\text{-}1^+$ $CD8^+$ T-cells to be more frequent in lymph nodes. $CXCR5^+PD\text{-}1^+$ $CD8^+$ T-cells resemble antigen-experienced memory-like cells, with high expression of KLRG1, CD127, granzyme K, and Eomes, and low expression of effector molecules like granzyme B and T-bet. They also express high levels of Tcf1, which is in accordance with the phenotype described in mice studies. We found $CXCR5^+PD\text{-}1^+$ $CD8^+$ T-cells within the EBV-specific T-cell population, but not within the FLU-specific T-cell compartment, showing that chronic antigens are required for this population to develop. To study their functionality, we are currently analyzing the transcriptome and functional capabilities of these cells in relation to ICB. These results can further elucidate the role for $CXCR5^+PD\text{-}1^+$ $CD8^+$ T-cells in clinical responses during ICB.

P.B4.01.08

TCR-activated T cells release cytokines that induce MDSC to express arginase-1, which does not suppress T cell proliferation

K. S. Kidder*;*
Georgia State University, Atlanta, United States.

Although previous reports suggest that tumor-induced myeloid-derived suppressor cells (MDSC) inhibit T cell proliferation by L-arginine depletion through arginase-1 activity, we herein show that arginase-1 is neither inherently expressed in MDSC nor required for MDSC-mediated inhibition of T cell proliferation. Employing Percoll density gradient centrifugation, large expansions of MDSC in the bone marrow of tumor-bearing mice were isolated and exhibit potent inhibition on T cell proliferation induced via TCR-ligation, Concanavalin A, PMA plus ionomycin, or IL-2. These MDSC, though suppressive toward T cell proliferation, do not hinder TCR-induced IFNγ secretion or granzyme B expression. Despite effectively inhibiting of T cell proliferation, both G- and M-MDSC exhibit no arginase-1 expression; however, arginase-1 can be induced by exposure to TCR-activated T cells or their culture medium, but not by T cells activated by other means or growing tumor cells. Western blot analysis revealed that TCR-activated T cells confer arginase-1 expression in MDSC by secreting cytokines that manifest as two distinct signaling-relay axes, IL-6-to-IL-4 or GM-CSF/IL-4-to-IL-10. Specifically, IL-6 signaling increases IL-4R on MDSC, enabling IL-4 to induce arginase-1 expression; similarly, GM-CSF in concert with IL-4 induces IL-10R, allowing IL-10-mediated induction. Surprisingly, our data demonstrate that the induction of arginase-1 is not conducive, per se, for MDSC-mediated inhibition of T cell proliferation, as neither supplementation of L-arginine nor arginase-1 inhibitor rescue T cell proliferation. Furthermore, this inhibition relies upon direct cell contact that is not dampened by PD-L1 blockade or SIRPα deficiency.

P.B4.01.09

CTLA-4 (CD152) impairs cytotoxic T-lymphocyte responses via PDCD4 induction

H. Lingel[1], *J. Wissing*[2], *A. Arra*[1], *F. Klawonn*[2], *M. Pierau*[1], *L. Jänsch*[2], *M. C. Brunner-Weinzierl*[1];
[1]*Otto-von-Guericke University, Magdeburg, Germany,* [2]*Helmholtz Centre for Infection Research, Braunschweig, Germany.*

Inhibitory T-cell surface receptors like Cytotoxic T-lymphocyte-associated Protein-4 (CTLA4) and Programmed cell death 1 (PD-1) play a crucial role in the termination of adaptive immune responses and promote the functionally impaired state of $CD8^+$ T cell exhaustion. Their blockade is being used in immune-checkpoint therapy as a promising approach to restore effective T-cell responses against tumors. However, the intracellular pathways triggered by these receptors still remain incompletely understood. To determine target molecules downstream of CTLA-4, an accurate mass spectrometry analysis was performed. The dataset revealed that the engagement of CTLA-4 led to altered phosphorylation of proteins involved in T-cell signaling, DNA replication, RNA processing and microtubule polymerization. Beside other targets, a CTLA-4-induced expression of the translational inhibitor Programmed cell death 4 (PDCD4) could be revealed and characterized. This mechanism was responsible for the restriction of cytotoxic T-lymphocyte effector functions. Accordingly, the deficiency of PDCD4 led to superior control of melanoma growth *in vivo*. Furthermore, identified upstream elements suggest this pathway as a part of a redundant mechanism that is activated by both CTLA-4 and PD-1. These findings point out that targeting of PDCD4 could provide a potent strategy to improve anti-tumor immunotherapy.

P.B4.01.10

Differentiation of CD8 T cells in the tumor microenvironment

A. Moustaki, *S. Alli, B. Youngblood;*
St Jude Children's Research Hospital, Memphis, United States.

Introduction: Differentiation of CD8 T cells to effector, memory, or exhausted state has been well characterized during viral infection, but it's poorly understood during tumorigenesis. Tumor infiltrating CD8 T cells reside in and are adapted to a unique, cellulary diverse and rapidly evolving environment, which delivers highly immunosuppressive signals. Here we investigate the role of tumor microenvironment in CD8 T cell activation in response to tumor antigens.
Methods: To monitor activation of tumor-specific CD8 T cells, we stably expressed the dominant MHCI-restricted LCMV-derived epitope GP33, in MC38 tumor cells. Co-expression of a reporter gene encoding for mCherry allows for detection of antigen presentation.
Results: Subcutaneous inoculation of MC38-GP33 cells into immunocompetent syngeneic hosts resulted in a massive expansion of highly functional CD8 effector cells in peripheral lymphoid tissues. However, GP33-reactive tumor infiltrating CD8 cells exhibit reduced polyfunctionality. Tumor cells genetically modified to express low levels of MHCI molecules were able to elicit a weakened immune response as manifested by the decreased frequency of tumor-reactive CD8 T cells. However, the reduced antigen presentation by tumor cells had no impact on the TME-induced dysfunction of CD8 T cells. Further characterization of MC38-GP33 tumors revealed the tumor infiltrating monocytes/macrophages as a source of tumor antigen cross-presentation, which coincidence with higher expression levels of PDL1 by the same cells.
Conclusions: Our findings highlight the importance of antigen and TME in the suppression of CD8 T cells function. We also identify tumor associated monocytes/macrophages as a potential driver of antigen-induced CD8 T cell exhaustion.

P.B4.01.11

Intratumoral CD4+ T cells with tissue-resident features resist exhaustion in non-small cell lung cancer

A. E. Oja[1], *B. Piet*[2], *D. van der Zwan*[1], *H. Blaauwgeers*[2], *M. Mensink*[3], *R. Stark*[1], *J. Borst*[3], *M. Nolte*[1], *R. van Lier*[1], *P. Hombrink*[1];
[1]*Sanquin Research, Amsterdam, Netherlands,* [2]*OLVG, Amsterdam, Netherlands,* [3]*The Netherlands Cancer Institute, Amsterdam, Netherlands.*

Resident memory T cells (T_{RM}) inhabit peripheral tissues and are critical for protection against localized infections. Recently, it has become evident that CD103 expressing T_{RM} are not only important in combating secondary infections, but also for the elimination of tumor cells. In several solid cancers, intratumoral CD103+CD8+ tumor infiltrating lymphocytes (TILs), with T_{RM} properties, are a positive prognostic marker. To better understand the role of T_{RM} in tumors, we performed a detailed characterization of CD8+ and CD4+ TIL phenotype and functional properties in non-small cell lung cancer (NSCLC). We found comparable levels of CD8+ and CD4+ T cell infiltrates in tumors, but observed a sharp contrast in T_{RM} ratios compared to surrounding lung tissue. While CD103+CD8+ T cells were enriched in tumors, CD103+CD4+ T cell frequencies were decreased when compared to surrounding lung tissue. In line with the immunomodulatory role of the tumor microenvironment, CD8+ and CD4+ TILs expressed high levels of co-inhibitory receptors, such as 2B4, CTLA4, and PD1, with the highest levels found on CD103 expressing TILs. Yet, CD103+CD4+ TILs proved to be the most potent producers of TNF-α and IFN-γ, while other CD4+ subsets and the majority of CD8+ TILs lacked such cytokine production. Furthermore, we found TILs with resident-memory features to express more costimulatory receptors, CD27 and CD28, when compared to lung T_{RM} suggesting a less differentiated phenotype. Agonistic triggering of these receptors improved cytokine production of TILs. Our findings provide a rationale to target CD103+CD4+ TILs to improve the efficacy of immunotherapies and cancer vaccines.

P.B4.01.12

Identification of potential immunological markers associated with the PD1 checkpoint blockade Nivolumab in non small cell lung cancer patients

S. Ottonello[1], *P. Carrega*[2], *I. Cossu*[3], *C. Genova*[4], *V. Fontana*[5], *F. Grossi*[4], *L. Moretta*[6], *M. Mingari*[7], *G. Pietra*[8];
[1]*Center of Excellence for Biomedical Research, Department of Experimental Medicine University of Genoa, Genoa, Italy,* [2]*Laboratory of Immunology and Biotherapy, Department of Human Pathology, University of Messina, Messina, Italy,* [3]*Immunology Unit, San Martino Hospital, Genoa, Italy,* [4]*Lung Cancer Unit, San Martino Hospital, Genoa, Italy,* [5]*Clinical Epidemiology Unit, San Martino Hospital, Genoa, Italy,* [6]*Immunology Area, Pediatric Hospital Bambino Gesù, Rome, Italy,* [7]*Center of Excellence for Biomedical Research, Department of Experimental Medicine University of Genoa, Immunology Unit, San Martino Hospital, Genoa, Italy,* [8]*Department of Experimental Medicine University of Genoa, Immunology Unit, San Martino Hospital, Genoa, Italy.*

PD-1 blockade represents a breakthrough in NSCLC therapy. However, to date robust biomarkers associated with response have not been identified. In this study we assessed whether particular immunological signatures evaluated over the course of treatment might be predictive of response to Nivolumab. We performed a deep phenotypic analysis in longitudinal blood samples of 73 advanced NSCLC patients treated with Nivolumab, collected at baseline and every 2 weeks before each therapy cycle. We examined the frequencies of several peripheral blood lymphocyte subpopulations ($CD4^+$ T cells, $CD8^+$ T cells, exhausted CD8+ T cells, NK cells) and their PD-1 expression. Cytometry data were then correlated with overall survival (OS) and clinical response as assessed by following RECIST. The higher % of $CD3^+$ T cells at baseline correlated with longer OS (*P =0.048*). In contrast, a baseline higher % of $PD1^+CD3^+$ T cells (*P =0.048)*, as well as of $PD1^+CD8^+$ T cells correlated with shorter OS (P=0.034). We next evaluated the frequency and the kinetics of exhausted $CD8^+$ T cells on therapy. Interestingly, we observed a lower frequency of exhausted $CD8^+$ T cells in pretreatment samples of PR+SD patients compared with PD patients (P=0.0462). In addition, in PR+SD patients, the frequency of exhausted $CD8^+$ T cells was reduced during treatment (from cycle 1 to 4) (P=<0.0001, P=0.0032, and P=0.0239, respectively*)*. Our results suggest that a higher % of PD-1 in T cells at baseline could correlate with shorter OS. Furthermore, the different amount of exhausted $CD8^+$ T cells could predict response and clinical outcome.

P.B4.01.13

Evaluation of potential factors contributing to the exhaustion of T lymphocytes in the tumor microenvironment

M. Rouault*, A. Laermanns, N. Li, J. Kim, X. Ma, E. Park;*
Advanced Cell Diagnostics, Newark, United States.

Immunosuppressive molecules and cells in the tumor microenvironment can lead to T cell dysfunction. CD8-positive cytotoxic T cells are ineffective in killing tumor cells primarily due to upregulated expression of inhibitory checkpoint molecules and decreased production of cytokines. Moreover, immune suppressive cell types and tumor associated macrophages are recruited to the TME, further establishing a suppressive immune environment. We evaluated expression profiles of immunosuppressive molecules and cells by applying RNAscope® assay and dual ISH-IHC staining. We evaluated CD8-positive cell infiltration in TME of human tissues from NSCL and ovarian cancer. Selected tissues with either high or low CD8-positive cell number were evaluated for (1) the presence of Tregs and TAMs, (2) the expression of immune checkpoint molecules PD1, PD-L1, TIM3, and LAG3, (3) the expression of immune suppressors IDO1 and TGFβ, and (4) IFNγ expression in CD8-positive subsets. CD8-high tissues expressed higher level of immune checkpoint molecules, while expression of IDO1 and TGFβ was independent of CD8-positive cell inflammation. Tregs and TAMs co-existed with IFNγ-positive CTLs in the same TME. PD1, TIM3, and LAG3 were expressed in tumor cells at lower level than in immune cells. Expression of IDO1 and TGFβ was observed in tumor cells. This study highlights the potential of RNAscope® ISH to understand the mechanisms associated with T cell dysfunction and exhaustion in TME. The RNAscope® platform is well suited for evaluating secreted factors in a cell type-specific manner and may facilitate the identification and development of biomarkers to stratify patients based on their specific cell states.

P.B4.01.14

HLA-DR in cytotoxic and regulatory T cells dictates breast cancer aggressiveness and patient response to neoadjuvant chemotherapy

D. P. Saraiva[1], *S. Braga*[1,2], *A. Jacinto*[1], *M. G. Cabral*[1,3];
[1]*CEDOC, NOVA Medical School, Lisbon, Portugal,* [2]*Instituto CUF de Oncologia, Lisbon, Portugal,* [3]*Faculdade de Engenharia da Universidade Lusófona, Lisbon, Portugal.*

Neoadjuvant chemotherapy (NACT) is the treatment option for locally advanced breast cancer (BC). However, approximately half of the patients have no response. To promptly direct non-responders to personalized therapies, there is an urgency to find a predictive biomarker of response. Tumor infiltrating lymphocytes are being appointed as biomarkers; nonetheless, tumor cells can escape the immune system, dampening CD8+ T cells (CTLs) and increasing regulatory T cells (Tregs) activation. HLA-DR, a T cell activation marker, can reflect the tumor-immune environment and be a reliable biomarker of NACT-response.
Fresh BC biopsies, surgical specimens and blood were immunophenotyped by flow cytometry. Peripheral blood mononuclear cells (PBMCs) were isolated and cultured under canonical stimuli.
BC with no metastasis in the lymph nodes have $HLA\text{-}DR^{hi}$ CTLs (p=0.003) and $HLA\text{-}DR^{lo}$ Tregs (p=0.002), when compared to node positive, although the average percentage of lymphocytes and myelocytes are similar between groups. Biopsies from NACT-responders have $HLA\text{-}DR^{hi}$ CTLs (p=0.0006) and $HLA\text{-}DR^{lo}$ Tregs (p=0.0002). HLA-DR+ CTLs upregulate IFN-γ, Granzyme B, Perforin, TNF-α and Eomes, essential for the effector function. $HLA\text{-}DR^{hi}$ CTLs negatively correlate with pro-tumorigenesis molecules - TGF-β, PD-L1, IL-6, IL-1β and IL-8 (p<0.005); while $HLA\text{-}DR^{hi}$ Tregs positively correlate with them and also with IL-10 (p<0.005). HLA-DR in tumor T cells correlates with its level in systemic T cells (CTLs: r=0.58, p=0.001; Tregs: r=0.65, p=0.0002). PBMCs stimulated *in vitro* from NACT-responders reveal higher IFN-γ and lower IL-10 (p<0.05).
HLA-DR levels in T cells can be used as biomarker of BC aggressiveness and NACT-response with the advantage of peripheral detection.

P.B4.01.15

Tumor-infiltrating Mucosa-Associated Invariant T (MAIT) cells express cytotoxic effector molecules and kill target cells

P. Sundström[1], *L. Szeponik*[1], *F. Ahlmanner*[1], *M. Sundquist*[1], *J. S. Wong*[2,3], *E. Bexe Lindskog*[4], *B. Gustavsson*[4], *M. Quiding-Järbrink*[1];
[1]*Dept of Microbiology and Immunology, Sahlgrenska Academy at University of Gothenburg, Sweden,* [2]*Department of Pathology, National University Hospital, Singapore, Singapore,* [3]*Department of Microbiology, National University of Singapore, Singapore,* [4]*Dept of Surgery, Sahlgrenska Academy at University of Gothenburg, Sweden.*

Mucosa-associated invariant T (MAIT) cells all express a semi-invariable T cell receptor recognizing microbial metabolites presented on the MHC class I-like molecule MR1. Upon activation, they rapidly secrete cytokines and increase their cytotoxic potential. We showed recently that MAIT cells accumulate in human colon adenocarcinomas, but that their ability to produce IFN-γ upon polyclonal stimulation is compromised. Here, we investigated the cytotoxic potential of MAIT cells in colon adenocarcinoma patients, and to what extent it may be affected by the tumor microenvironment. MAIT cells were identified by flow cytometry and analyzed for their expression of cytotoxic effector molecules and degranulation. Polyclonal, T cell receptor-, and cytokine-mediated activation of MAIT cells from tumors induced increased Granzyme B, while degranulation was mainly seen in response to cognate antigen recognition. The cytotoxic potential of tumor-associated MAIT cells was similar to that of MAIT cells from unaffected colon. Furthermore, tumor infiltrating pre-activated MAIT cells killed antigen-presenting target cells. MAIT cells were also identified by immunofluorescence in direct contact with tumor cells in sections from colon cancer specimens. Taken together, our data demonstrate that tumor-associated MAIT cells from colon tumors have potent cytotoxic function and are not compromised in this regard compared to MAIT cells from the unaffected colon. We conclude that MAIT cells may contribute significantly to the protective immune response to tumors, both by secretion of Th1-associated cytokines and by direct killing of tumor cells.
Funding sources: Swedish Research Council and Swedish Cancer Foundation.

P.B4.01.16

A novel CD3/BCMA bispecific antibody for the treatment of multiple myeloma and plasma cell disorders selectively activates effector T cells with improved safety

*M. Farinacci[1,2,3], K. Juelke[1,2,3], B. Buelow[4], **W. vanSchooten**[4], R. Buelow[4], H. Volk[2,3];*
[1]Core Unit Immunocheck, Charite University of Medicine, Berlin, Germany, [2]Institute for Medical Immunology, Charite´ University of Medicine, Berlin, Germany, [3]Berlin-Brandenburg Center for Regenerative Therapies, Charite´ University of Medicine, Berlin, Germany, [4]Teneobio Inc., Menlo Park, United States.

Introduction: Multiple myeloma (MM) is a cancer of plasma cells, and the second most common hematological malignancy. A promising therapeutic in development for MM is TNB-383B, an antiCD3*BCMA bispecific antibody. This T cell engager combines high tumor specificity (anti-BCMA arm) with a selective novel T cell activating anti-CD3 arm. Efficacious T cell engagers with limited toxicity will allow combination therapies with other immune-modulators in MM and expansion into non-malign "plasma cell" disorders such as several autoimmune diseases.
Material and methods: TNB-383B was tested in standard in vivo and in vitro tumor models. To assess the potential risk of cytokine release syndrome (CRS) human peripheral blood mononuclear cells from healthy volunteers (n=3) were pre-incubated for 48 hours at high densities and, subsequently, incubated with increasing concentrations of TNB-383B overnight in the presence of recombinant BCMA protein coated to plastic. OKT3 and a T cell engaging bispecific with a high-affinity anti-CD3 arm were used throughout these experiments as positive controls.
Results: Both TNB-383B and the positive control T cell engager (PC) mediated tumor cell lysis in in vitro and animal tumor models. In vitro analysis of upregulation of CD69 and CD137 on T cells showed that TNB-383B, in contrast to PC, preferentially activated $CD4^+$ and $CD8^+$ T effector cells relative to $CD4^+$ T regulatory cells. Remarkably, T-cell activation by TNB-383B was associated with low cytokine production compared to PC.
Conclusions: TNB-383B represents a novel immunotherapeutic with potentially improved safety and efficacy for the treatment of MM and pathogenic long-lived plasma cells.

P.B4.01.17

Prognostic implications of tissue resident memory T cells in human melanoma development

***M. Willemsen**[1,2,3], G. Krebbers[1,2,3], F. R. Kasiem[1], T. R. Matos[1,3], R. Luiten[1,2,3];*
[1]Academic Medical Center, Amsterdam, Netherlands, [2]Cancer Center Amsterdam, Amsterdam, Netherlands, [3]Amsterdam Infection & Immunity Institute, Amsterdam, Netherlands.

Tissue-resident memory T (T_{RM}) cells permanently reside in epithelial barrier tissues and can respond rapidly upon reinfection. Recently, expression of the retention integrin very late antigen (VLA)-1, by vaccine-induced T cells was found to correlate with longer patient survival in melanoma. More interestingly, VLA-1 was frequently co-expressed with tissue residence markers, such as CD69 and CD103. Furthermore, CD103-dependent T_{RM} cells seem to play a key role in sustaining immunity to melanoma, indicating a crucial function for T_{RM} cells in antitumor immunity. Yet, its role in melanoma development remains unknown and might be relevant, for there are numerous neoplastic lesions in the skin that rarely become overt cancers. This research, therefore, aimed to identify the prognostic relevance of skin resident memory T cells in human melanomagenesis.
Healthy skin, naevocellulares nevi, dysplastic nevi, lentigo maligna, lentigo maligna melanoma, nodular primary melanoma, superficially spreading primary melanoma and cutaneous metastatic melanoma (all n=7) were analyzed by immunohistochemistry for the presence of T_{RM} cells. The prognostic significance of CD103 and VLA-1 expression on T_{RM} cells was also investigated.
The presence of T_{RM} cells may serve as prognostic marker for disease progression. Furthermore, intratumoral T_{RM} cells, especially in primary melanoma, may potentially be a good effector in cancer immunotherapies.

P.B4.01.18

General outlook of the immune surveillance status in oncourological patients

***P. Yakovlev**[1], D. Klyushyn[2];*
[1]O.Bogomolets National Medical University, Kyiv, Ukraine, [2]Taras Shevchenko National University of Kyiv, Kyiv, Ukraine.

Cancer initiation, progression and immune surveillance recruit lymphocytes as common key cellular players. The progression of the cancer occurs against the immunocompromised status in the patient. Much attention is being paid to the cellular branch of immunity in cancer patients either as marker or therapeutic target. Aim. To analyze the status of the immune surveillance system in patients with urinary tract cancer. Materials and methods. The retrospective analysis of pretreatment immunogram of 90 patients staged I-IV cancer of urinary bladder, kidney and prostate scheduled for definitive surgical treatment at the department of urology in 2015-2018 was performed. Levels of lymphocytes CD3+, CD4+, CD8+, CD3+56+16+, CD3-56+16+, CD19+, CD14+, and immune-regulatory index were compared to normal range disregard of the cancer stage. Results. Majority of immune cell parameters fell below the normal range: CD14+ were lower in 80% of patients, CD19+ in 76%, CD4+ in 42%, CD3+ in 40%, CD3+56+16+ in 20%, CD8+ in 15%, CD3-56+16+ in 12%, and IRI - in 40%. Elevation in immune cells content was observed: with CD3+56+16+ in 15%, CD8+ in 12%, and CD3-56+16+ in 12% of patients, mostly in advanced cancer patients. Conclusion. Vast deficiency in B-cell immunity was observed in majority (76%) of urological cancer patients, and prevails such of the T-cell branch. The T-cells quantitative fluctuations present the diverse pattern, with elevation above normal range of cytotoxic, NK-cells, and T-suppressors mostly in patients with advanced urinary cancer.

P.B4.01.19

Evaluation of autologous tumor-antigen specific $CD8^+$ T-cell responses to $BRAF^{V600E}$ melanoma cells in the course of MAPKi treatment

*N. Pieper[1], F. N. Harbers[1], A. Zaremba[1], M. Schwamborn[1], S. Lübcke[2], B. Schrörs[2], A. Sucker[1], V. Lennerz[2], T. Wölfel[2], D. Schadendorf[1], A. Paschen[1], **F. Zhao**[1];*
[1]University Hospital Essen, Essen, Germany, [2]University Medical Center, Johannes Gutenberg University Mainz, Mainz, Germany.

Expressed in approximately 50% of melanomas, the V600 mutation in the *BRAF* gene promotes tumor proliferation via constitutive activation of BRAF-MEK-ERK in the MAPK signaling pathway. Significant clinical responses in patients with advanced metastatic melanoma can be achieved by targeting this axis using MAPK inhibitors (MAPKi), such as BRAFi and MEKi as single or combination therapy. But over time the majorities of treated patients acquire resistance and experience disease progression. Salvage therapies for resistant patients often involve immunotherapies, e.g. immune checkpoint blockade to reinvigorate tumor-reactive T cells and generate sustained clinical responses. However, the impact of MAPKi on melanoma immunogenicity over time remains largely unknown. For the development of an optimal treatment protocol, we evaluated the recognition of MAPKi-exposed melanoma cells by autologous tumor infiltrating $CD8^+$ T-cells *in vitro* and regularly observed the evolution of melanoma resistance to the pre-existing tumor-specific T-cell repertoire during prolonged BRAFi treatment in different patient models. While efficiently recognizing short-term BRAFi-treated melanoma cells, TILs were less responsive towards long-term exposed tumor cells. Similar results were obtained with four out of five $CD8^+$ T-cell clones against three shared antigens and one mutated neoantigen due to a time-dependent downregulation of their respective target antigens. Moreover, T-cell resistance evolved not only after mono-treatment of melanoma cells with BRAFi but also after combined BRAFi/MEKi exposure. Our observation that T cell-resistance coevolves with drug-resistance in melanoma cells suggests that BRAFi application preceding T cell-based immunotherapy might be disadvantageous.

P.B4.01.20

Multifactorial contribution to follicular helper bias in the CD4 T cell response to a retroviral antigen

***L. Danelli**, G. Kassiotis;*
The Francis Crick Institute, London, United Kingdom.

The extent of CD4 T cell differentiation during an immune response is influenced by multiple intrinsic and extrinsic factors resulting in the generation of a diverse array of T helper (Th) cell phenotypes. Here, we analysed the response of CD4 T cells reactive with a murine retroviral envelope glycoprotein model antigen and identified an uncommonly strong bias towards follicular helper (Tfh) commitment and impaired Th1 differentiation in its natural context during retroviral infection. Comprehensive quantitation of variables known to influence CD4 T cell fate decision suggested that Tfh differentiation closely correlates with persistent TCR signalling, irrespective of TCR clonotypic avidity. However, strong TCR signalling leading to TCR downregulation and induction of LAG3 expression further restrained CD4 T cell commitment and differentiation. The response to the same antigen in the context of altered lymphocyte alteration and in different immunisation regimens generated a variable balance of Tfh and Th1 cells, suggesting that the stunted Th1 differentiation could be due to limited IL-2 availability during retroviral infection. These results highlight the potent contribution of T cell extrinsic variables in determining the relative balance of Tfh and Th1 responses according to the nature of the antigenic stimulus.

P.B4.01.21

Control of cytotoxic lymphocyte anti-tumor functions through CD226 expression

*M. Weulersse, A. C. Pichler, M. Joubert, H. Avet-Loiseau, **L. Martinet**;*
Cancer Research Center of Toulouse, Toulouse, France.

Although $CD8^+$ T lymphocytes activation mainly relies on TCR recognition of antigenic peptides presented by MHC-I, numerous signals transmitted through activation and inhibitory receptors impact their functions. Promising results obtained by immune checkpoint blockers in cancer highlight the necessity of defining receptors that control cytotoxic lymphocyte anti-tumor reactivity. CD226 (DNAM-1) is an adhesion molecule stimulating $CD8^+$ T cell cytotoxicity against target cells expressing its ligands, CD112 and CD155. Despite the recent defects associated with the absence of this molecule in several malignancies, the molecular role of CD226 in cytotoxic T lymphocyte biology remains poorly understood to date. We recently identified two distinct CD8+ T cells populations based on CD226 expression. We found that unlike the $CD226^+$ counterparts, the $CD226^-$ $CD8^+$ T cells population have functional defects in response to TCR stimulation including lower proliferation, pro-inflammatory cytokine production and cytolytic activity. Global transcriptional analysis and gene transfer experiments revealed that $CD8^+$ T cells effector program initiated upon TCR stimulation requires CD226 expression.

Indeed, CD226 absence leads to reduced phosphorylation of key downstream TCR signaling proteins and lower calcium influx highlighting an unsuspected role for CD226 in TCR signaling. Finally, we found that CD226$^-$ T cells with reduced effector functions accumulate in Human cancer patients and that CD226 loss may be involved in immune exhaustion and cancer escape. References : Martinet et al, Nature immunology, 2014. Martinet et al, Cell Reports, 2015. Martinet et al, Nature Reviews Immunology, 2015 . Guillerey ….and Martinet, Journal of Clinical Investigation, 2015

P.B4.02 T-cell activation and exhaustion - Part 2

P.B4.02.01

Flow cytometry as a diagnostic tool to detect cellular and functional defects in patients with primary immunodeficiencies

***M. Bitar**, A. Boldt, U. Sack;*
Institute of Clinical Immunology, Leipzig, Germany.

Primary immunodeficiency diseases (PIDs) are genetic disorders, that mostly cause susceptibility to infections and are sometimes associated with autoimmune and malignant diseases. Mutations can affect cells and molecules of the innate as well as the adaptive immune system (T and/or B cells). Here, we present the use of flow cytometry in the diagnostic cascade to identify possible PID. First, simple immunophenotyping of B-, NK-, CD4+, CD8+, double negative T cells and analysis of HLA-DR/CD38 is recommended to identify severe abnormalities in lymphocyte subsets e.g. in Brutons Disease or SCID. Further differentiation (naïve, memory, recent thymic emigrants, activation markers etc.) of T-, B- and NK-cell subsets in a second step is necessary to differentiate PID with defects in the subsets such as CVID or DiGeorge syndrome and many others. Based on that well defined immunophenotyping, functional analysis of leukocytes can be performed in a third step.
For instance, flow cytometry is a useful tool for measurement of (1) T- and B-cell proliferation in case of different forms of lymphopenia or unclear deficiencies in lymphocyte subsets, (2) phagocytosis/oxidative burst in chronic granulomatous disease, (3) loss-of-function STAT1 mutation and chronic mucocutaneous candidiasis CMC (gain-of-function gene mutations in STAT1), (4) STAT3 phosphorylation to distinguish between different Hyper-IgE syndrome (HIES) traits (autosomal dominant with STAT3 mutation in contrast to autosomal-recessive without STAT3 mutation).
In conclusion, flow cytometry is well-standardized and a flexible method for investigation of most relevant leukocyte subsets and their immune function. Therefore, FCM should be considered as an important tool in PID-diagnostics.

P.B4.02.02

Function of T-bet and Eomes in CTLs - an evolutionary approach

***J. Fixemer**[1], J. F. Hummel[1], T. Kögl[2], K. Weißert[2], C. Klose[3], S. Arnold[4], P. Aichele[2], Y. Tanriver[1];*
[1]Institute of Medical Microbiology and Hygiene, University Medical Center Freiburg, Freiburg, Germany, [2]Institute of Medical Microbiology and Hygiene, Department of Immunology, Freiburg, Germany, [3]Jill Roberts Institute for Research in Inflammatory Bowel Disease, Cornell University, New York, United States, [4]Institute of Experimental and Clinical Pharmacology and Toxicology, Freiburg, Germany.

The two T-box transcription factors *Tbx21* (encoding for T-bet) and *Eomesodermin* (encoding for Eomes) have arisen from duplication of a single ancestral gene. In contrast to their ancestor both have various functions in adaptive immunity indicating a benefit for the host throughout evolution. Usage of knock-out mice for T-bet or Eomes revealed redundant and non-redundant functions in T cells. However, recent studies emphasized that transcription factors act in highly context and cell specific networks.
To dissect the individual contributions of T-bet and Eomes to the differentiation of T cells we developed a novel mouse model. We used TALEN technology to knock-in *Eomesodermin* into the first exon of *Tbx21* to ensure the expression of *Eomesodermin* under complete transcriptional control of *Tbx21*.
By infecting *Tbx21*$^{+/+}$, *Tbx21*$^{-/-}$ and *Tbx21*$^{Eomes/Eomes}$ mice with 200 pfu LCMV WE we use FACS technology to compare transcriptional activities of *Tbx21* and *Eomesodermin* on the protein level in T cells at day 8 and 30 post infection. We point out that T-bet is essential for expansion and cytotoxicity of CTLs and that Eomes compensates poorly for T-bet on an overall level in T cells. In conclusion our data shed light on the evolutionary pressure leading to the genesis of T-bet as a master regulator of type 1 immune responses.

P.B4.02.03

TLR2 and TLR7-mediated co-stimulation differently enhance cytokine production of cytotoxic T cells

***J. J. Freen-van Heeren**, F. Salerno, A. Guislain, M. Wolkers;*
Sanquin Research, Amsterdam, Netherlands.

Optimal T cell activation requires antigen recognition through the T cell receptor (TCR), engagement of costimulatory molecules, and cytokines. T cells can also directly recognize pathogen associated molecular patterns through the expression of toll-like receptors (TLRs). Whether TLR ligands can provide costimulatory signals and enhance antigen-driven T cell activation is not well understood. Here, we show that TLR2 and TLR7 ligands potently lower the antigen threshold for cytokine production in T cells. To investigate how TLR triggering supports cytokine production, we adapted the protocol for flow cytometry-based fluorescence *in situ* hybridization (Flow-FISH) to mouse T cells. The simultaneous detection of cytokine mRNA and protein with single-cell resolution revealed that TLR triggering primarily drives *de novo* mRNA transcription. *Ifng* mRNA stabilization only occurs when the TCR is engaged. TLR2- but not TLR7-mediated costimulation can potentiate mRNA stability at low antigen levels. Importantly, TLR2 costimulation also increases the percentage of polyfunctional T cells. In conclusion, TLR-mediated costimulation effectively supports and potentiates T cell effector function.

P.B4.02.04

Effect of transition metal containing particulate matter (TMCPM) on the activity of human immune cells

***A. Gałuszka**, M. Stec, M. Siedlar, J. Baran;*
Department of Clinical Immunology, Institute of Pediatrics, Jagiellonian University Medical College, Cracow, Poland.

In recent years, significant increase in air pollutions seriously impacts the human health. Growing evidence supports the role of various environmental agents in the breakdown of tolerance leading to inflammatory reaction via numerous mechanisms. The aim of our study was to determine the effect of TMCPM on antigen presenting capacity of monocytes and production of pro-inflammatory cytokines, as well as on the activity of CD4+ T cell subsets.
Two different preparations of TMCPM were used in the study: NIST (SRM 1648a- standard urban particulate matter) and LAP (NIST 1648a treated with a low-temperature plasma). Antigen presenting capacity of monocytes, production of reactive oxygen species (ROS) and pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF), mitochondrial membrane potential and viability of monocytes were examined after exposure to TMCPM. In case of CD4+ T lymphocyte subpopulations (Th1, Th2, Th17, Treg), their relative amount (percentage) was evaluated after culture with TMCPM.
Decreased capacity of monocytes to present antigen to T lymphocytes and increased production of pro-inflammatory cytokines and ROS induced by TMCPM has been observed. Production of ROS caused decrease of mitochondrial membrane potential and lowers viability of monocytes. In case of T lymphocytes, the level of Th1 and Th17 cells increased, while the percentage of Treg cells decreased after exposure to TMCPM.
TMCPM treatment alters antigen presenting capacity of human monocytes, induces production of proinflammatory cytokines and upsets the balance of Th1/Th2 and Treg/Th17 cells, promoting pro-inflammatory activity of Th subsets.
This study was supported by grant from the NCN in Poland no. 2015/16/WST5/00005.

P.B4.02.05

Regulation of innate-like T cell activation by IL-32

***J. P. Hagel**[1], K. Powell[1], H. Mehta[1], P. Phalora[1], T. Leng[1], F. M. Buffa[2], P. Klenerman[1,3], C. B. Willberg[1,3];*
[1]The Peter Medawar Building for Pathogen Research, University of Oxford, Oxford, United Kingdom, [2]Computational Biology and Integrative Genomics, Department of Oncology, University of Oxford, Oxford, United Kingdom, [3]NIHR Biomedical Research Centre, University of Oxford, Oxford, United Kingdom.

Natural Killer (NK) and innate-like T cells, including the mucosal-associated invariant T (MAIT) cell subset, share the ability to respond to cytokines such as IL-12 and IL-18. A novel pluripotent cytokine, IL-32, has been reported to modulate NK cell responses to cytokine-induced activation, but its effect on MAIT cells is currently unknown.
We show IL-32 can significantly increase cytokine-mediated IFNγ- and Granzyme B-expression in CD161++ T cells, which includes the MAIT cell subset, within PBMCs and isolated CD8+ T cells. However, it does not have any measurable effect on TNFα-expression. Moreover, IL-32 promotes cytokine-mediated upregulation of checkpoint markers, including LAG3, PD1 and TIM3. The observed IL-32-mediated effects are time- and dose-dependent. No significant effect of IL-32 on TCR-mediated responses was detected. Blocking IL-32 during cytokine- or *E.coli*-mediated MAIT cell activation, within PBMCs, decreased MAIT cell responses significantly, indicating a crucial role for IL-32 in MAIT cell activation. Furthermore, IL-12+IL-18 stimulation of PBMCs and CD3+ T cells resulted in the expression of IL-32 by CD161++ T cells. This identifies a previously undescribed function of MAIT cells. In summary, our data show that IL-32 can promote cytokine-mediated responses of CD161++ T cells, including the MAIT cell subset. These cells are also the predominant subset of T cells expressing IL-32 in response to IL-12 and IL-18, indicating a potential feedback loop. Our findings broaden the understanding of cytokine- and bacterial-mediated activation of MAIT cells and potentially provide another reference point for future clinical intervention in disease.

P.B4.02.07

Acid Sphingomyelinase deficiency reduces cytotoxic activity of CD8+ T cells during melanoma progression

M. Hose[1], *A. Westendorf*[1], *J. Buer*[1], *A. Haimovitz-Friedman*[2], *W. Hansen*[1];
[1]*Institute of medical microbiology, University Hospital Essen, Germany,* [2]*Memorial Sloan Kettering Cancer Center, New York City, United States.*

Acid sphingomyelinase (Asm) is a hydrolyzing enzyme and part of the sphingolipid metabolism. After activation Asm converts sphingomyelin to ceramide at the outer leaflet of the plasmamembrane. Self-accumulating properties of ceramide lead to the generation of ceramide-enriched platforms. These platforms are involved in receptor clustering and thereby in intracellular signal transduction. Tumor cells were shown to have a double growth rate when implanted into Asm-deficient mice as compared to wildtype mice. The role of T cells in this differential tumor progression is still elusive. Therefore we transplanted B16-F1 melanoma cell lines s.c. into Asm-deficient mice and analyzed the activation status of tumor infiltrating lymphocytes. Moreover, we depleted different T cell subsets to dissect their specific function in our experimental setting. Our results indicate that tumor infiltrating T cells in Asm-deficient mice are less activated. Moreover, we could show that Asm-deficiency in CD8+ T cells lead to an impaired cytotoxic activity and shifts them to a suppressive phenotype within the tumor tissue. In summary our study reflects the importance of Asm abundance in T cells during melanoma progression.

P.B4.02.08

Analysis of signaling pathways triggered by costimulatory receptor GITR

Š. Janušová*, H. Draberova, A. Drobek, O. Stepanek, P. Draber;*
Institute of molecular genetics, Praha, Czech Republic.

T cell immune responses are initiated upon the recognition of an antigen presented on MHC glycoproteins by specific T cell receptors. However, for proper immune response, additional costimulatory signals are required. Several members of the TNF-receptor superfamily, such as GITR, CD27, CD137 or OX40, can provide the required costimulatory signaling, which affects T cell survival, proliferation, and effector functions. These receptors are expressed on activated T cells and, after engagement with their ligands, they trigger downstream signaling events, such as activation of NF-κB, p38 or JNK signaling pathways. The precise understanding how the costimulatory signaling is propagated would provide a potential target(s) to modulate T cell responses in autoimmune diseases or cancer. In this study we analysed molecular mechanisms modulating signaling after GITR stimulation. We aimed to find novel molecules participating in these pathways and elucidate how they affect GITR-induced signaling responses.

P.B4.02.09

TCR and inflammatory signals tune human MAIT cells to exert specific tissue repair and effector functions

T. Leng*, T. King, C. Willberg, P. Klenerman;*
Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom.

Background: Human mucosal associated invariant T (MAIT) cells are present in circulation as well as immune privileged sites such as the liver and the gut. They are a homogenous innate T cell subset with regard to their phenotypic and functional properties. Studies have illustrated an array of MAIT cell effector functions, ranging from MR1-mediated antibacterial responses to TCR-independent, cytokine-mediated antiviral properties, also proinflammatory roles in autoimmune diseases.
Aim: We aim to examine the impact of combinations of TCR-dependent and -independent signals in blood and tissue-derived human MAIT cells, and to extend our knowledge on the functional properties of this tissue-resident T cell subset.
Conclusion: We show that while TCR triggering is insufficient to drive full activation, gene expression signatures of TCR-triggered MAIT cells showed specific enrichment of tissue-repair functions (e.g. TNF, FURIN, VEGFA, CSF1). Activation of certain effector and antimicrobial functions - measured by IFN-γ release and Granzyme B expression – may be triggered further with specific innate cytokine signals, such as the TNF superfamily member TL1A. These features were reproduced in gut-derived cells. By genome-wide comparison of functional responses to TCR-dependent and independent signals, we defined novel functions of MAIT cells relevant not only to host defence but also tissue homeostasis.
Discussion: Our study suggests that MAIT cells could serve a potential therapeutic target in IBD treatment, where reducing local inflammation and promoting tissue repair is essential. We hope to gain deeper insight into relevant functions of MAIT cells by investigating transcriptional signatures of gut-derived MAIT cells.

P.B4.02.10

A segment of amino acids of LAT adaptor modifies its turnover and has a dual role in TCR intracellular signaling

M. Martinez de Arbulo-Echevarria[1], *I. Narbona-Sanchez*[1], *C. Fernandez-Ponce*[1], *I. Vico-Barranco*[1], *J. Muñoz-Miranda*[1], *M. Rueda-Ygueravide*[2], *M. L. Dustin*[3], *A. Miazek*[4], *M. Duran-Ruiz*[1], *F. Garcia-Cozar*[1], *E. Aguado-Vidal*[1];
[1]*University of Cádiz, Cadiz, Spain,* [2]*Neumology and Alergic diseases, Hospital Puerta del Mar, Cadiz, Spain,* [3]*The University of Oxford, Headington, United Kingdom,* [4]*Institute of Immunology and Experimental Therapy, Wroclaw, Poland.*

Normal T cell development and function requires TCR/CD3 signaling that proceeds through the activation of protein tyrosine kinases and phosphorylation of downstream molecules. The membrane-anchored adaptor Linker for Activation of T cells (LAT) has an essential role transducing intracellular signals coming from the TCR/CD3 complex. It has been shown that upon T-cell activation LAT interacts with the tyrosine kinase Lck, leading to the inhibition of its kinase activity. The segment comprising residues 112-126 of human LAT is required for its interaction with Lck, and this domain is rich in negatively charged amino acids and is conserved among different species. In order to deepen into the functional relevance of LAT-Lck interaction, we have substituted this segment of LAT with a non-charged segment of amino acids and expressed this mutant LAT (LAT-NIL, from Not Interacting with Lck) in LAT deficient J.CaM2 cells. Substitution of this segment in LAT does not alter its expression in the plasma membrane, but prevented the activation-induced interaction with Lck. LAT-NIL mutant shows a reduced stability with regard to WT-LAT after cycloheximide treatment. J.CaM2 cells expressing this mutant form of LAT showed a statistically significant increase of proximal intracellular signals, such as phosphorylation of LAT in tyrosine residues 171 and 191, and also enhanced ZAP70 phosphorylation approaching borderline statistical significance ($p = 0.051$). Nevertheless, downstream signals such as Ca^{2+} influx or MAPK pathways were partially inhibited. Overall, our data reveal that LAT-Lck interaction constitutes a key element regulating proximal intracellular signals coming from the TCR/CD3 complex.

P.B4.02.11

Cell type dependent activation of Mucosal associated invariant T cells in the Liver

H. K. Mehta[1,2], *C. B. Willberg*[1,2], *P. Klenerman*[1,2,3];
[1]*University of Oxford, Oxfordshire, United Kingdom,* [2]*Peter Medawar Building for Pathogen Research, University of Oxford, Oxfordshire, United Kingdom,* [3]*Oxford NIHR Biomedical Research Centre, The John Radcliffe Hospital, Oxfordshire, United Kingdom.*

Mucosal associated invariant T (MAIT) cells are enriched within the Liver representing a major hepatic T cell subset. However, the ability of diverse liver parenchymal cells to interact with MAIT cells has not been well explored. We investigated the capacity of different hepatic cell lines to activate MAIT cells in response to both bacteria and viruses. Immortalized primary hepatocyte (IPH) (HHL12), hepatocellular carcinoma (HCC) (HepG2 and Huh7), stellate (TW-NT) and liver sinusoidal endothelial cell (LSEC) lines were used to stimulate MAIT cells in the presence of E.coli or hepatotropic viruses. MAIT cell activation was analysed by flow cytometry. The HCC lines, HepG2, Huh7 and IPH line, HHL12 and stellate cell lines were not able to stimulate MAIT cells after exposure to E.coli or viruses. However, the LSEC cell line is able to readily activate MAIT cells for cytokine production (IFN-γ and TNF-α). In addition to this we observed an increase in GrB production from MAIT cells upon activation. LSECs activate MAIT cells primarily through the MR1 pathway but also utilise the alternative cytokine pathway (IL-12/IL-18). HBV-infected LSECs partially activated specific MAIT cell functions. In conclusion, through their anatomical location and interaction with pathogens, together with their functional activity as antigen presenting cells, LSECs may be critical activators of MAIT cells in the liver for bacterial and potentially for viral infections.

P.B4.02.12

Differences in ERAP1 allotype function correlate with HPV epitope processing, TIL status and prognosis in HPV-positive OPSCC

E. Reeves*, E. King, G. Thomas, T. Elliott, E. James;*
University of Southampton, Southampton, United Kingdom.

Human papillomavirus (HPV) infection accounts for 5% cancers worldwide, with cervical carcinoma and oropharyngeal squamous cell carcinoma (OPSCC) being most common. Levels of tumour infiltrating lymphocytes (TIL) in HPV+ cancers are an indicator of prognosis and survival, with fewer TIL having a worse clinical outcome. Polymorphisms in the endoplasmic reticulum aminopeptidase1 (ERAP1), a key component of antigen processing trimming N-terminally extended peptides for MHC I, are associated with prognosis and outcome in cervical carcinoma. We have previously demonstrated ERAP1 to be highly polymorphic, forming distinct allotypes with functional differences. Here we investigate the contribution of ERAP1 in OPSCC. We identified a number of ERAP1 allotype combinations (co-dominantly expressed chromosomal copies) from HPV+ OPSCC patients. Assessment of trimming function revealed a range of abilities to generate the model epitope SIINFEHL from individual amino acid precursors. Further analysis revealed that the activity of ERAP1 from TIL^{high} and TIL^{low} patients correlates with the property of the amino acid extension. Importantly, the ability of ERAP1 to generate the HPV-16 $E7_{82-90}$ epitope (LLMGTLGIV) from predicted precursors also correlated with TIL status. ERAP1 allotype pairs identified in TIL^{low} patients were poor at generating the final epitopes (SIINFEHL and LLMGTLGIV), whereas those identified in TIL^{high} patients generated the epitopes efficiently. These data reveal that ERAP1 gene sequence and function stratifies with TIL levels and prognosis, suggesting that the successful presentation of HPV-16 epitopes at the cell surface, and thus a strong anti-HPV T cell response, depends on the ERAP1 allotype pairs expressed within an individual.

P.B4.02.13

Assessment of CD320 expression and vitamin B12 function on human T-cells

***R. Schmidt**, A. Dopler, M. Gerner, L. Ziegler, K. Schmetterer;*
Department of Laboratory Medicine, Vienna, Austria.

Backgorund: Vitamin B12 is an essential co-factor for proliferating and metabolically active cells. After uptake into the organism, it is bound to transport proteins in the serum. Transcobalamine II is the transport protein that delivers Cobalamine to peripheral cells, where it binds to CD320, the Transcobalamin II receptor. Vitamin B12 deficiency presents with failure of highly proliferating and metabolically active tissue resulting in megaloblastic anemia or neurological dysfunctions. The effects of vitamin B12 on activation and function of lymphocytes have not been fully investigated so far.
Methods: T-Cells were negatively isolated from peripheral blood derived PBMC and polyclonally stimulated using antiCD3/antiCD28 coated beads. Following activation, mRNA levels and protein concentration of CD320 were measured by immunoblotting and flow cytometry. For functional assays, binding of TCII to CD320 was blocked using a monoclonal antibody.
Results: Upon activation, CD320 levels were up-regulated, with the highest expression after 72h. In the presence of anti-TCII antibody, the proliferation of T-Cells decreased while early activation markers, such as surface expression of CD69, CD25 and CD71 and production/secretion of IL-2 were not influenced. Similarly, TCR-proximal signaling events, such as phosphorylation of the CD3-zeta chain and the adapter molecules LAT and SLP-76 were also not influenced by the presence of anti-TCII.
Conclusion: These observations indicate that CD320 is an activation marker on T-cells. Furthermore, Vitamin B12 is essential for long-term T-cell activation/proliferation while activation steps preceding the induction of proliferation are independent of vitamin B12.

P.B4.02.14

PD-1/PD-L1 expression in malignant hematological diseases

*A. Dodopoulos, K. Psarra, **E. Grigoriou**, G. Papagiannopoulou, A. Tsirogianni, C. Tsigalou;*
Immunology-Histocompatibility Dept. "Evaggelismos" General Hospital, Athens, Greece.

Patients suffering from malignant hematological diseases, often fail to reach full remission. T lymphocyte dysfunction, as the result of inhibitory receptor expression, could be one of the causes.
The expression of PD-1 on normal lymphocytes as well as the expression of PD-L1 on patient's malignant cells was studied using flow cytometry. The group of patients consisted of 73 people (5 ALL, 18 AML, 10 MDS, 7 MM, 13 NHL and 20 CLL-MBL patients). 10 normal subjects consisted the PD-1 control group. PD-L1/PD-1 expression was assessed with a 5 and 7 color protocol respectively.
PD-1 expression was increased significantly on T lymphocytes concerning ALL, AML, MDS and NHL patients. MM had the strongest and CLL the weakest PD-1 expression. In NHLs, positive correlation was shown regarding PD-1 expression on CD4 and CD8 cells as well as NK and CD8 T cells in MM and ALL. MDS were the only group with a higher PD-1 expression in all lymphocytes subpopulations, compared to the control group. PD-L1 showed a unique expression pattern among groups. ALL showed the highest and MM the lowest expression. PD-L1 levels are significantly elevated on malignant cells compared to lymphocytes. But, no correlation was found between patient's PD-1 and PD-L1 levels. PD-1/PD-L1 expression using flow cytometry might be used for prognosis assessment for these diseases.

P.B4.02.15

Molecular transport at the immunological synapse controls T cell responses

***M. van Ham**[1], N. Amsberg[1], L. Philipsen[2], S. Kliche[2], J. Niemz[1], L. Simeoni[2], C. Falk[3], A. Müller[2], J. Hühn[1], B. Schraven[2], L. Jänsch[1];*
[1]Helmholtz Centre for Infection Research, Braunschweig, Germany, [2]Otto von Guericke University, Magdeburg, Germany, [3]Hannover Medical School, Hannover, Germany.

The assembly and activity of the TCR signalling network at the immunological synapse (IS) requires molecular transport of signalling microvesicles (MV). Following TCR activation, MVs are rapidly recruited along IS-projecting microtubules by the dynein motor complex. Interestingly, molecular transport at the IS discriminates Tconv- and Treg cells. We reported a subset-specific TCR network assembly and defined involved cytoskeleton regulators by phopshoproteomics. Among those we identified the dynein light intermediate chain 1 (LIC1), a dynein motor subunit that regulates cargo selection.
To analyse the role of LIC1 in T cell activation, component recruitment and IS formation we generated LIC1-deficient Jurkat T cells ($LIC1^{-/-}$ cells). General T cell functions including proliferation, adhesion, migration and TCR surface expression were not affected. Upon TCR engagement, however, $LIC1^{-/-}$ cells indicated aberrant phosphorylation dynamics at proximal components. Conjugation with Raji B cells revealed enhanced accumulation of an Lck phosphovariant at the IS. In addition, activated $LIC1^{-/-}$ cells exhibited reduced secretion of selected cytokines. Thus, the dynein motor subunit LIC1 seems to act as a TCR-dependent regulator for the selected adaptation of TCR signalling and cytokine releases. A better understanding of the regulatory network underlying MV molecular transport at the IS might pave the way for novel checkpoints to regulate TCR signalling of T cell subsets *in vitro* and *in vivo*.

P.B4.02.16

Role of diacylglycerol kinase alpha in X-linked lymphoproliferative disease 1 (XLP1) and autoimmunity

***S. Velnati**[1,2], E. Ruffo[3], A. Massarotti[4], M. Talmon[5], S. V. Konduru[1,2], A. Gesu[4], L. G. Fresu[5], A. L. Snow[6], G. C. Tron[4], A. Graziani[3], G. Baldanzi[1,2];*
[1]Department of Translational Medicine, university of piemonte orientale, Novara, Italy, [2]Institute for Research and Cure of Autoimmune Diseases, Novara, Italy, [3]School of Medicine, University Vita e Salute San Raffaele, Milan, Italy, [4]Department of Pharmaceutical Science, University of Piemonte Orientale, Novara, Italy, [5]Department of Health Sciences, School of Medicine, University of Piemonte Orientale, Novara, Italy, [6]Department of Pharmacology and Molecular Therapeutics, Uniformed Services University of the Health Sciences, Bethesda, United States.

X-linked lymphoproliferative disease 1 (XLP1), a primary immunodeficiency due to mutations in SLAM adaptor protein (SAP). SAP deficiency results in constitutive diacylglycerol kinase alpha (DGKα) activity, that decreases TCR signaling and impairs restimulation-induced cell death (RICD) of CD8+ T cells. Indeed, pharmacological inhibition of DGKα restores RICD in cellular model of XLP1 and limits CD8+ accumulation associated immunopathology in XLP1 animal models suggesting the development of DGKα inhibitors for XLP1 therapy. Alternatively, we promote RICD resistance by treating normal lymphocytes with osteopontin (OPN), a matrix protein that is known to promote lymphocyte proliferation and migration. Interestingly, DGKα inhibitors restore RICD also in OPN treated cells, indicating a major DGKα role in the OPN signaling and as a general regulator of RICD sensitivity. Finally, to find new DGKα inhibitors suitable for human use, we used a 2D/3D in silico approach based on chemical homology with the two commercially available DGKα inhibitors (R59922 and R59949). Out of the resulting 127 compounds, ritanserin (serotonin antagonist) and compound01 (uncharacterised molecule) were highly specific for DGKα and showed equal or superior potency compared to R59022 and R59949. In cellular models of XLP-1, both ritanserin and compound01 restored RICD of SAP-deficient CD8+ without significant toxicity. Furthermore, compound01 doesn't perturb serotonin signaling, thus represent a lead compound for further development. Indeed, we are executing compound optimization program to find compound01 derivatives.
Our findings may contribute to the development of innovative therapies for diseases characterized by RICD resistance such as XLP-1 but also in autoimmunity.

P.B4.02.17

Use of melanin from yeast-like fungies *Nadsoniella nigra* sp. X1 (i. Galindez, Antarctica) in surgically treated urological stage IV cancer patients

***P. Yakovlev**[1], T. Falalyeyeva[2], L. Garmanchuk[2], T. Beregova[2], L. Ostapchenko[2];*
[1]O.Bogomolets National Medical University, Kyiv, Ukraine, [2]Taras Shevchenko National University of Kyiv, Kyiv, Ukraine.

Patients with stage IV cancer require combined treatment approach to achieve good outcomes. Surgery is always supplemented with adjuvant treatment. Supportive medicinal care plays crucial role in correcting immunosuppressed state due to cancer itself and further worsened by the surgical intervention, to enable patients the faster recovery to proceed with adjuvant treatment. Purpose: Assess the effect of melanin on immune surveillance parameters of stage IV urological cancer patients after definitive treatment. Materials and methods. During 2015-2018 forty patients with stage IV urological cancer (19 - bladder cancer, and 21 - kidney cancer) were subjected to extirpative surgery with postoperative systemic treatment. Postoperatively patients took "Melatal" pills (Melanin 10g) twice a day during 30 days. Blood analysis and immunogram were collected prior, after surgery, and after 30 days of Melatal intake. Controls were 20 patients with similar stage cancer subjected to same surgery but with no adjuvant "Melatal". Results. All study patients demonstrated significant decline below the norm on white blood cell count and immunogram, in particular: lower leucocytes (2.7-3.5*10*3/ml), lymphocytes (0.6-1.0*10*3/ml), CD4+ cells (0.1-0.22*10*3/ml, 13-20%), IRI (0.2-0.6), CD3+56+16+ (1.2%), CD3-56+16+(1.9-3.2%), CD19+ (1.7-6.3%), CD14+ (2-4%). One month after surgery, patients after "Melatal" had better total leucocytes (5.1 vs. 3.5*10*3/ml), CD4+ (35% vs. 28%), IRI (1.3 vs. 0.9), CD14+ (9% vs. 6%), CD19+ (12% vs. 9%) than control patients. Conclusion. Intake of polyphenol compound Melanin during one month in the adjuvant regimen in patients with stage IV urological cancer after extirpative treatment yields beneficial effect in strengthening the parameters of immune surveillance.

P.B4.02.18

Signal 3 cytokines promote effector-associated epigenetic programs during mouse and human memory CD8 T-cell differentiation

***C. C. Zebley**, H. Abdelsamed, H. E. Ghoneim, A. Moustaki, S. Alli, M. Wehenkel, M. McGargill, B. Youngblood;*
St. Jude Children's Research Hospital, Memphis, United States.

Recent evidence suggests that the CD8 T-cell subset of stem cell memory (T_{scm}) cells has an enhanced ability to eradicate tumor and is therefore considered an ideal subset for chimeric antigen receptor (CAR) T-cell therapy. Consequently, significant interest exists in determining how long-lived memory T-cells, such as T_{scm}, are generated. We recently reported that human memory T-cell differentiation is associated with the acquisition of DNA methylation programs that promote effector function. Using these epigenetic signatures, we assessed the differentiation status of currently established CAR T-cell protocols. Surprisingly, we found current protocols for *in vitro* differentiation of T_{scm} from naïve CD8 T-cells do not instill cells with effector-associated DNA methylation programs. Given the epigenetic profiles of *in vitro* generated T_{scm} do not reflect those of a bona fide T_{scm} we assessed the requirement of signal 3 cytokine co-stimulation for inducing effector-associated epigenetic programs during memory CD8 T-cell differentiation. Varying *in vitro* culture cytokines induces changes in the phenotype of naïve human CD8 T-cells which are reflected at the epigenetic level. For instance, *in vitro* culture conditions involving IL-12 result in demethylation at the IFNγ locus with corresponding increase in IFNγ expression. Furthermore, *in vivo* experiments with coinfection of C57BL/6 mice with Listeria monocytogenes and LCMV Armstrong induce an inflammatory environment that results in epigenetic poising of the downstream IFNγ locus. Demethylation of the IFNγ locus likely results from infection-induced cytokines. These data demonstrate that cytokines can be used to engineer an epigenetically defined memory T-cell population poised to recall effector functions.

P.B4.02.19

In vitro induction of CTL exhaustion

***M. Zhao**, C. J. Stairiker, M. van Meurs, Y. M. Mueller, P. D. Katsikis;*
Erasmus Medical Center, Rotterdam, Netherlands.

Exhaustion is a dysfunctional state of CTL during chronic infection and cancer. Establishing an in vitro system that induces CTL exhaustion could greatly facilitate the study of this phenotype and allow the testing of novel approaches to reverse exhaustion. Chronic antigen stimulation has been proposed as a critical contributor to CTL exhaustion. To simulate chronic antigen stimulation, purified splenic OT-1 CD8+ T cells from transgenic mice were cultured with cytokines and were repeatedly stimulated with SIINFEKL peptide. On day 5, cells were harvested and inhibitory receptors and cytokine production after restimulation with peptide were assayed by flow cytometry. Cells were also rested for 3 or 5 days and reassessed for functions. After 5 days of repeat peptide stimulation, there was significant upregulation of inhibitory PD-1 and Lag-3 expression on CTL. Furthermore, the production of IL-2 and TNF-α was reduced, while IFN-γ was unaffected. Additionally, these cells expanded less than controls. After resting without peptide, the high expression of inhibitory receptors was maintained, while cytokine deficiencies were partially recovered. Further validation of these cells in vivo is under investigation. In summary, we have established an in vitro system to induce CTL exhaustion. Such a system will facilitate the exploration of exhaustion reversing strategies.

P.B4.02.20

Deletion of Map4k4 enhances CD8 T cell function

***E. Esen**;*
Genentech, San Francisco, United States.

Map4k4 is a serine threonine kinase of the STE20 family that is expressed broadly. It is highly expressed in immune cells. We investigated the role of Map4k4 in CD8 T cell function. Deletion of Map4k4 induces CD8 T cell cytokine production, activation, proliferation and target killing *in vitro*. Map4k4 iKO (induced knock-out) animals showed reduced tumor growth when MC38 cells were injected subcutaneously. In the tumors, T cells expressed less exhaustion markers and produced more inflammatory cytokines when stimulated ex vivo. We showed that CD8 T cells were both necessary and sufficient for reduced tumor growth difference in the Map4k4 iKO animals. Using LCMV-Armstrong infection model, we showed that there is increased antigen specific CD8 T cell production in the iKO animals and these CD8 T cells showed enhanced cytokine production. We also showed elevated activation markers using OT1 transgenic model. To sum up, Map4k4 deletion leads to enhanced CD8 T cell proliferation and activation. Mechanistically, we found that Map4k4 kinase activity is necessary for its role in CD8 T cells and it signals through ERM family of proteins.

P.B4.03 T-cell activation and exhaustion - Part 3

P.B4.03.01

Visualizingthe immune response of the T helper cells to the osteopathic manipulativetreatment in normal subjects

***A. Abdelfattah**, N. Abdelraoof, S. Nasef;*
Faculty of physical therapy - Cairo University, Cairo, Egypt.

Purpose: This study was designed to investigate the Effect of selected osteopathic lymphatic techniques on immune system in healthy subjects. Method: Forty five subjects (33 males and 12 females), with age ranged from 20 to 30 years old participated in this study. They were assigned into three equal groups each one has 15 subjects: group A received sternal pump and sternal recoil techniques .Group B received thoracic lymphatic pump and splenic pump techniques for 12 sessions, three sessions per week. Group C (control group) did not receive any physical therapy modality. Absolute count of CD4 and WBCs count were used to evaluate participants before and after the study. Results: Statistical analysis revealed that there was a significant increase in CD4 P value was ≤ 0.045 and WBCs count P-value was ≤ 0.006 between before and after treatment with the second group in the two experimental groups. While there was no significant difference in the same measuring variables in the first and control groups. Comparison between groups revealed that there was a significant difference between the first and second groups in CD4 and WBCs, P: probability< 0.05. Conclusion: The second osteopathic manipulative treatment group was the effective method of enhancing the immune system in healthy subjects (thoracic lymphatic pump (TLPT) and splenic pump techniques (SPT). Key words: Osteopathy, CD4, Thoracic lymphatic pump, splenic pump technique, Sternal pump technique and Sternal recoil technique. Ethical committee approval: Cairo university:P.T.R.E/012/00945 ANZCTR NO: ACTRN12616000216415

P.B4.03.02

The transcription factor TOX controls differentiation and maintenance of T cells in chronic infection

***F. Alfei**, K. Kanev, P. Roelli, M. von Hoesslin, M. Wu, D. Zehn;*
Technische Universität München (TUM), Freising, Germany.

Introduction: During chronic infections, virus-specific CD8 T cells acquire a differentiation program that is distinct from the one acquired in acute infections. In this case, the persisting antigen stimulation causes T cells to become dysfunctional which means that they show impaired effector functions and up-regulated inhibitory receptor expression. To understand the molecular foundation of CD8 T cells dysfunction, we compared the transcriptional profiles of normal and dysfunctional CD8 T cells. We found the transcription factor TOX as one of the mostly upregulated gene in chronically stimulated CD8 T cells.
Materials and methods: To study the function of TOX, we generated a conditional KO mouse which facilitates peripheral deletion of functional TOX protein in P14 T cells. Following adoptive transfer of KO or WT P14 in naïve mice, we monitored their behave in LCMV Armstrong or clone13 infections. Flow cytometry, NGS, immunofluorescence and immunohistochemistry analysis were performed.
Results: The absence of TOX impairs the survival of dysfunctional CD8 T cells, without affecting CD8 T cells baring an acute phenotype. This impaired survival goes along with a loss of the critical TCF-1 expressing population, decreased PD-1 but increased KLRG1 expression and augmented cytokine production. The resulting enhanced effector phenotype leads to a better viral clearance but also to an increase in immunopathology.
Conclusions: TOX promotes the dysfunctional phenotype in CD8 T cells, nonetheless ensures their fitness for long term maintenance during chronic infection.

P.B4.03.03

Innocent Bystanders: Chronic Virus Infection Compromises Memory Bystander T cell Maintenance and Function

***I. Barnstorf**[1], N. Baumann[1], M. Borsa[1], K. Pallmer[1], A. Yermanos[1], N. Joller[2], S. P. Welten[1], N. J. Krautler[1], A. Oxenius[1];*
[1]ETH Zurich, Zurich, Switzerland, [2]University of Zurich, Zurich, Switzerland.

Chronic viral infections are widespread among humans, with approximately 8-12 chronic viral infections per individual and there is epidemiological proof that these impair heterologous immunity. We studied the impact of chronic virus infections on the phenotype and function of memory bystander CD8 T cells. Active chronic virus infection had a profound effect on total numbers, phenotype and function of memory bystander T cells in mice. The phenotypic changes included upregulation of markers commonly associated with effector and exhausted cells and were induced by IL-6 in a STAT1-dependent manner in the context of chronic virus infection. Furthermore, bystander CD8 T cell functions were reduced with respect to their ability to produce inflammatory cytokines and to undergo secondary expansion upon cognate antigen challenge with major cell-extrinsic contributions responsible for the diminished memory potential of bystander CD8 T cells. These findings open new perspectives for immunity and vaccination during chronic viral infections**.**

P.B4.03.04

Distinct function of the co-signaling partners BTLA and HVEM on the virus-specific CD8+ T cells response during LCMV infection

***P. Diethelm**, I. Schmitz, J. Kisielow, M. Kopf;*
Institute of Molecular Health Sciences, Zurich, Switzerland.

Activation of naïve T cells is controlled through engagement of co-stimulatory and co-inhibitory molecules. It has been reported that binding of HVEM (Herpes Virus entry mediator) to BTLA (B and T lymphocyte attenuator) inhibits T cell expansion however recent evidence suggests that BTLA can serve reciprocally as an activating ligand for HVEM. By infecting single and double KO animals with an acute or a chronic dose of LCMV we made the unexpected finding that neither effector anti-viral T cell responses nor memory formation were impaired. Accordingly, no difference in virus clearance was found between knockout and control wild-type animals. Interestingly, staining of BTLA and HVEM on gp33-specific CD8+ T cells at the peak of the immune response revealed that both molecules were down regulated implicating that this may promote T cell expansion. Indeed, retroviral overexpression of HVEM on virus-specific CD8+ T cells resulted in reduced numbers of effector cells 7dpi. In contrast, BTLA overexpression rather increased the cytokine production of gp33-specific CD8+ T cells. Whether sustained expression of HVEM affects proliferation or survival of anti-viral CD8+ T cells during the effector phase and whether the cytotoxic activity of BTLA overexpressing CD8+ T cells is increased is currently under investigation. Together these data imply that HVEM down regulation on gp33-specific CD8+ T cells ensures efficient accumulation 7dpi and thus control of an LCMV infection whereas down regulation of BTLA dampens the cytokine response of anti-viral CD8+ T cellsand thus limits inflammation.

P.B4.03.05

Bacillus Calmette-Guérin (BCG) causes Vgamma9 Vdelta2 T-cell proliferation and potentiates cytotoxic responses towards tumour cells

***J. R. Fenn**[1], R. Reljic[1], S. Sharpe[2], M. D. Bodman-Smith[1];*
[1]St. George's, University of London, London, United Kingdom, [2]Public Health England, Salisbury, United Kingdom.

Vγ9Vδ2 T-cells can recognise malignantly transformed cells as well as those infected with mycobacteria. This cross-reactivity supports the idea of using mycobacteria to manipulate Vγ9Vδ2 T-cells in cancer immunotherapy. To date, therapeutic interventions using Vγ9Vδ2 T-cells in cancer have involved expanding these cells in or ex vivo using zoledronic acid (ZA). In this study we show that the mycobacterium-based vaccine, Bacillus Calmette-Guérin (BCG), also causes Vγ9Vδ2 T-cell expansion in vitro. We phenotypically and functionally compared Vγ9Vδ2 T-cells expanded using ZA to those expanded using BCG and compared the ability of these two populations of Vγ9Vδ2 T-cells to kill myeloid THP-1 target cells. We show that ZA expanded Vγ9Vδ2 T-cells kill ZA pre-treated THP-1 cells but are unable to target untreated THP-1 cells. Conversely, BCG expanded Vγ9Vδ2 T-cells are able to target both ZA treated and untreated THP-1 cells. Furthermore, BCG expanded Vγ9Vδ2 T-cells were able to effectively kill BCG infected THP-1 cells whereas ZA expanded Vγ9Vδ2 T-cells were not. These data suggest that BCG treatment could be a way of generating Vγ9Vδ2 T-cells with greater tumouricidal potential compared to ZA treatment and that either ZA or BCG could be used intratumourally as a means to potentiate stronger anti-tumour Vγ9Vδ2 T-cell responses.

P.B4.03.06

Molecular imaging of the antigen recognition dynamics in CD8+ cytotoxic T-cells

***M. Kraller**, R. Platzer, P. Spechtl, J. Huppa, H. Stockinger;*
Medical University of Vienna, Vienna, Austria.

Cytolytic T-cells (CTLs) can detect with their low affinity T-cell antigen receptors (TCRs) the presence of even a single antigenic peptide-loaded MHC molecule I (pMHCI) among thousands of structurally related yet non-stimulatory pMHCs (Purbhoo et al. 2004). How they achieve this is not clear but appears to depend at least in part on the special binding conditions within the special constraints of the immunological synapse, the area of contact between a T-cell and an antigen presenting cell. Here receptors and their ligands are not only pre-oriented, but they are often enriched in specific membrane domains and also subjected to cellular forces. To relate these cell biological parameters to T-cell antigen sensitivity in a more comprehensive manner we are monitoring TCR-pMHC binding in nascent synapses with the use of molecular imaging modalities. We confront TCR transgenic CTLs with a glass-supported lipid bilayer (SLB) functionalized with pMHCI, adhesion and co-stimulatory molecules. This allows us to conduct (single molecule) measurements in noise-attenuated Total Internal Reflection (TIRF) mode, to control for ligand composition and density to quantitate their specific influence on TCR-pMHCI binding and TCR-proximal downstream signaling. We also plan to assess the role of CD8 co-receptor engagement with the use of pMHCI mutants, which are deficient in CD8 binding. In this fashion we expect to gain novel insights into cell biological and molecular processes underlying the phenomenal sensitivity of CTLs towards antigen.

P.B4.03.07

Analyzing chimeric single chain antigen receptors by using a triple parameter T cell reporter line

*J. Brunner[1], **J. Leitner**[1], D. A. Boardman[2,3], G. Lombardi[2,3], J. Maher[4], M. H. Heemskerk[5], W. Paster[6], P. Steinberger[1];*
[1]Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria, [2]MRC Centre for Transplantation, King's College London, Guy's Hospital, NIHR Biomedical Research Centre, Guy's & St Thomas'NHS Foundation Trust & King's College London, Guy's Hospital, London, United Kingdom, [3]NIHR Biomedical Research Centre, Guy's & St Thomas'NHS Foundation Trust & King's College London, Guy's Hospital, London, United Kingdom, [4]CAR Mechanics Group, Division of Cancer Studies, King's College London, Guy's Hospital, London, United Kingdom, [5]Department of Hematology, Leiden University Medical Center, 2300 RC Leiden, Netherlands, [6]Department of Clinical Cell Biology and FACS Core Unit, Children's Cancer Research Institute (CCRI), Vienna, Austria.

Adoptive transfer of genetically modified T cells expressing single-chain antigen receptors like chimeric antigen receptors (CAR) or chimeric single-chain TCR (cTCR) are emerging and highly promising approaches for cancer treatment. Within this study different signaling domains for such receptors were assessed in a Jurkat triple parameter transcriptional cell line 76 (Jurkat 76 TPR). This T cell reporter line allows to simultaneously assess the activity of three transcription factors that play essential roles in T cell activation processes - NF-kappaB, NFAT and AP-1. A CAR recognizing HLA-A2 and the HLA-A2 restricted cTCR X15 specific for the human T cell lymphotrophic virus (HTLV) Tax peptide were used as model single-chain antigen receptors. Both were fused to signaling domains derived from 4-1BB, CD28 and CD3ζ to generate signaling modules that correspond to first, second and third generation CARs. The resulting reporter lines were stimulated with engineered antigen-presenting cells (APC). Assessment of reporter gene expression by flow cytometry showed that activation of Jurkat reporter cells critically depended on the signaling modules contained in the single-chain antigen receptors. Direct comparison of 4-1BB and CD28 as costimulatory modules in both types of receptors (CAR and cTCR) demonstrated a much higher capacity of the 4-1BB domain to induce reporter gene expression. Our results indicate that transcriptional T cell reporter cells are a powerful mean to assess signaling domains for single-chain antigen receptors.

P.B4.03.08

The role of Interleukin-18 on the phenotypical and functional plasticity of cytotoxic T cells in pancreatic cancer

***V. Lutz**, A. Hanlon, E. Landmann, M. Buchholz, T. Gress, C. Bauer;*
Clinic for Gastroenterology, Endocrinology, Metabolism and Infectiology, Philipps-University Marburg, Marburg, Germany.

Introduction: $CD8^+$ cytotoxic t cells (CTL) play a central role in tumor response mechanisms. In pancreatic cancer CTL show decreased activation. Mechanistically, proinflammatory cytokines, like IL-1 and IL-18, might play a crucial role in the induction of this dysfunctional state. This project investigates the role of IL-18 on cytotoxic T cell responses in a murine model of pancreatic cancer.
Methods: Antigen-specific CTL were generated from transgenic OT-I mice and from transgenic OT-I mice crossed with IL-18R-KO mice. CTL were co-cultured with antigen-loaded dendritic cells (DC), in the absence or presence of IL-18 and IL-1. As a parameter of effector function the intracellular production of IFN-γ and TNF was determined. OT-I or OT-IxIL-18R-KO CTL were adoptively transfected in PancOVA tumor bearing mice. CTL were analyzed for exhaustion markers, degranulation efficiency and intracellular production of IFN-γ and TNF.
Results: IL-1 enhances CTL effector function, whereas IL-18 does not influence IFN-γ production of CTL. Interestingly, IL-18R-deficient T cells demonstrate increased effector activation when stimulated with IL-1 in co-culture with antigen-loaded DC in comparison to WT-CTL. However, stimulation of DC with IL-18 prohibits increase of IFN-γ production in WT-CTL.
Conclusions: In the next steps we intend to characterize the potentially suppressive signaling pathway of IL-18. The role of IL-18 in the immune response against pancreatic cancer will be determined further by examining the effect on non-t cells, tumor cells and stroma cells on phenomena of T cell exhaustion in an *in vivo* model.
This project is funded by von Behring-Röntgen-Stiftung.

P.B4.03.09

Utility of *STAT3* and *STAT5b* mutations as diagnostic and prognostic tool in T/NK large granular lymphocyte leukemia (LGLL)

N. Muñoz-García[1,2,3], *M. Jara-Acevedo*[1,2,3], *C. Caldas*[1,2,3], *P. Bárcena*[1,2,3], *A. López*[1,2,3], *N. Puig*[4,2,3], *M. Alcoceba*[4,2,3], *N. Villamor*[5,6,7], *P. Fernández*[8], *M. González*[9,2,3], *A. Orfao*[1,2,3], *J. Almeida*[1,2,3];

[1]*IBMCC (University of Salamanca), Salamanca, Spain,* [2]*IBSAL, Salamanca, Spain,* [3]*CIBERONC, Salamanca, Spain,* [4]*University Hospital of Salamanca, Salamanca, Spain,* [5]*Hospital Clínic, Barcelona, Spain,* [6]*IDIBAPS, Barcelona, Spain,* [7]*University of Barcelona, Barcelona, Spain,* [8]*Kantonsspital, Aarau, Switzerland,* [9]*University Hospital of Salamanca and USAL, Salamanca, Spain.*

Introduction: the clinical utility and significance of *STAT* mutations in T-cell LGLL (T-LGLL) and NK-cell chronic lymphoproliferative disorders (CLPD-NK) remains uncertain. Here, we analyzed the frequency of somatic mutations in *STAT3* and *STAT5b* genes in clonal LGLL expansions from diverse T/NK-cell lineages and its relationship with biological (phenotype) and clinical characteristics of the disease.
Materials and Methods: *STAT3* and *STAT5b* genes were sequenced in 157 populations of FACS-sorted cells (previously phenotyped by multiparametric flow-cytometry), from patients with 97 monoclonal and 7 oligoclonal LGL expansions, 17 non-cytotoxic T-CLPD and 36 polyclonal T-cell populations.
Results: *STAT3* or *STAT5b* somatic mutations were detected in 30/97 populations from patients with clonal LGL expansions (31%), all but one in the *STAT3* gene. According to the LGLL cell lineage, mutations were present in 31% of TαβCD8$^+$, 32% of CLPD-NK, 7% of TαβCD4$^+$CD8$^{-/+d}$, 100% of TαβCD4$^+$CD8$^+$, 50% of TαβCD4$^-$CD8$^-$ and 44% of Tγδ expansions. *STAT3* was also detected in 1/17 non-cytotoxic T-CLPD, but in none of polyclonal cytotoxic T-cell populations. Two patients who had ≥2 clonal LGL expansions from different cell-lineages showed *STAT3* mutations in at least two of them. Interestingly, patients carrying *STAT* mutated LGL expansions showed a significantly higher incidence of autoimmune disorders (vs. non-mutated monoclonal LGL expansions), supporting a prognostic value.
Conclusions: we support the utility of *STAT3*/*STAT5b* mutations for clonality assessment and prognostic evaluation in T-LGLL and CLPD-NK; additionally, these findings suggest that similar activation pathways would be involved in the pathogenesis of LGLL, regardless of the cytotoxic cell-lineage involved.
IBSAL grant #IBPredoc17/00012

P.B4.03.10

Identification of two human CD8$^+$ T cell subsets with distinct cytokine profile

B. P. Nicolet*, A. Guislain, F. van Alphen, M. van den Biggelaar, M. C. Wolkers;*
Sanquin Research, Amsterdam, Netherlands.

CD8 T cells are critical to kill virally infected and tumor cells. Intriguingly, when activated, human CD8 T cells show a dichotomy in cytokine production. Whereas some T cells produce only IFNg, others are found to make specifically IL-2, or they produce both cytokines. Whether this cytokine profile is fixed or whether it is dependent on kinetics and oscillation is not to date known. Here we developed a method that allowed us to perform proteomics analysis on fixed, FACS-sorted T cells based on their cytokine expression profile. This analysis revealed that a distinct expression profile between the cell types. Whereas IL-2 single producing cells display a helper-like phenotype, the IFNg-single and double producing T cells show a cytotoxic profile with a high co-expression of TNFa, granzymes, perforin, granulysin and chemokines. Furthermore, we identified several surface markers that can be used to enrich for the different cytokine producers. Using this approach, we found that the cytokine profile was robust over a prolonged culture period of the cells. In conclusion, we identified two distinct T cell populations in human T cells that exert differential functions. This may be exploited for T cell-mediated therapies.

P.B4.03.11

A rapid, high throughput multiplex assay that measures T-cell activation, cytokine secretion, and identifies T-cell subsets from multiple donors

J. O'Rourke*, Z. Liu;*
Intellicyt Corporation, A Sartorius Company, Albuquerque, United States.

The identification of T-cell subsets and assessing their *ex vivo* activation is a key step in the adoptive cell therapy process. To facilitate this workflow, we developed a high throughput flow cytometry-based, multiplexed assay to measure T cell activation in different T-cell subsets. PBMCs from multiple donors were profiled prior to and after T cell enrichment with CD3/CD28 magnetic beads. Purified T cells were cultured in the presence of the beads and media containing IL-2 for 3 days and cell phenotypes and secreted cytokines were analyzed. For each sample well, 11 cytokines and 13 cellular endpoints were measured each day over the course of a three-day activation protocol including quantifying cells expressing the activation markers CD69, CD25 and HLA-DR. A 96-well sample plate contained T cells from 12 different donors, with each sample analyzed in quadruplicate using two different sample dilutions. The plates were read on the iQue Screener Plus and the high-content data was analyzed using the integrated ForeCyt software. The results indicate a time course dependent, donor to donor variation in T-cell activation. These high throughput, large-scale flow cytometry studies provide extensive T cell activation and subset profiles from multiple donors with assay times of ~15 minutes. The adoption of these assays can provide actionable data to optimize adoptive cell therapy culture conditions and improve manufacturing protocols.

P.B4.03.12

Evaluation of helper T cell proliferation in the co-cultures of lung adenocarcinoma cells, neutrophils and monocytes

F. G. OZBAY*, G. ESENDAGLI;*
Hacettepe University Cancer Institute Department of Basic Oncology, Ankara, Turkey.

T cell responses are modulated especially by antigen presenting cells; on the other hand, in the inflammatory microenvironment of lung adenocarcinoma, these cells have been shown to be located nearby neutrophils. Hence, this study aims to investigate helper T (Th) cell proliferation in a co-culture setting containing lung adenocarcinoma cells, neutrophils and monocytes.
Peripheral blood CD66b+ neutrophils, CD14+ monocytes, and CD4+ T cells were purified from the healthy volunteers with density gradient centrifugation, followed by MACS and/or FACS procedure. These cells were co-cultured at 1:1:1 ratio in the presence of anti-CD3 mAb. A549 and H1299 were used as lung adenocarcinoma cell lines. On a flow cytometry, T-cell proliferation was assessed by eFluor670 dilution assay, cell viability by propidium iodide exclusion, neutrophil and monocyte activation by CD62L, CD66b, CD11b, CD86, HLA-DR expression.
The presence lung cancer cells and monocytes supported the viability of neutrophils and inhibition of ROS production leaded to higher T cell proliferation in the co-cultures. Neutrophils were in an activated state with decreased CD62L and increased CD11b levels.
In a co-culture setup, which was established to partially model the tumor microenvironment, employing neutrophils, monocytes, Th-cells and lung cancer cells, our initial findings show the importance of neutrophils as a critical modulator for Th-cell responses.

P.B4.03.13

Mapping of T-cell responses in dengue exposed individuals from India

E. Reddy[1], *S. Gunisetty*[2], *K. Nayak*[1], *M. Gupta*[1], *H. Panda*[1], *M. Singla*[3], *R. Lodha*[3], *S. Kabra*[3], *P. Coshic*[3], *M. Kaur*[1], *S. Yazdani*[i], *M. Kaja*[1], *A. Chandele*[1];
[1]*International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India,* [2]*Emory Vaccine Centre, ATLANTA, Georgia,* [3]*All India Institute of Medical Sciences (AIIMS), New Delhi, India.*

Dengue is a global epidemic and T cells have been implicated in both protection and pathogenesis of dengue infections. Thus far, a handful of studies have *in silico* predicted and tested CD4 and CD8 T cell epitopes but none have comprehensively characterized dengue-specific CD4 and CD8 T cell epitopes *ex vivo*.
In this study, using overlapping peptides spanning the entire proteome of the dengue 2 virus, we performed a detailed analysis to identify immune epitopes recognized by dengue-specific memory CD4 and CD8 T cells. Because of low precursor frequency of memory T cells, we employed a methodology of approach of long-term peptide and IL-2 stimulation of PBMC's derived from dengue-seropositive and seronegative individuals, followed by restimulation and assessment of peptide-specific proliferation and IFNγ production by flow cytometry.
Corroborating with previous reports, we observe CD4 T cell specificities are distributed across all dengue proteins. In contrast the CD8 T cell specific responses are typically conserved to the non-structural and viral envelope. By combining knowledge of subject specific HLA and their IFNγ producing antigenic regions, we have identified 117 CD8 T-cell epitopes and 240 CD4 T-cell epitopes. A majority of these dengue 2 epitopes are conserved in dengue 1, 3 and 4. However, interestingly, up to 10% of epitopes are cross conserved in other Flavivirues, like Zika, JEV, YFV and WNV.
Taken together, our study shows breadth of both CD4 and CD8 T cell responses found *in vivo* to the entire dengue proteome and opens novel implications in dengue T-cell cell-based vaccine.

P.B4.03.14

***In vivo* TCR signaling during chronic viral infection**

***I. Sandu**, D. Cerletti, A. Oxenius, M. Claassen;*
ETH, Zürich, Switzerland.

A hallmark of chronic infections is exhausted CD8 T cells, characterized by a perturbed transcriptional program, upregulation of inhibitory receptors, impaired effector function, reduced numbers, and alteration of normal memory development, driven by prolonged T cell receptor (TCR) engagement.
This project focuses on characterizing the kinetics of TCR signaling in different tissues in mice chronically infected with Lymphocytic Choriomeningitis virus. TCR signaling was quantified *in vivo* using virus-specific transgenic CD8 T cells expressing a reporter under the control of the *Nur77* promoter.
Our data reveals that, despite available antigen *in vivo*, NUR77 expression is very low in exhausted cells. In addition, these cells express multiple co-inhibitory receptors known to inhibit TCR signaling. Indeed, both blockade of the major inhibitory PD-1-PDL1 pathway and transfer of target cells lacking ligands binding co-inhibitory receptors into chronically infected mice increased NUR77 reporter levels temporarily.
Preliminary results of single-cell RNAseq performed on exhausted cells isolated from various organs revealed distinct subpopulations, suggesting that tissue microenvironment has a major impact on shaping the structure, phenotype, and function of these cells.
Thus, persistent TCR triggering during chronic infection leads to a near complete shut-down of *in vivo* TCR signaling, largely due to the combined action of inhibitory receptors, explaining the poor ability of exhausted cells to kill endogenous virus-infected targets. However, cytotoxic potential is preserved because they can efficiently kill transferred target cells lacking ligands for inhibitory receptors. Moreover, single-cell transcriptomics reveals that exhausted cells are heterogeneous and adopt tissue-specific signatures.
Project funded by ETH grant 39_14-2

P.B4.03.15

Phenotypic and functional characteristics of HER2-specific cytotoxic T lymphocytes activated by DNA-transfected dendritic cells and enriched by magnet sorting and cytokine stimulation

*M. Kuznetsova, J. Lopatnikova, **S. Sennikov**;*
Research Institute of Fundamental and Clinical immunology (RIFCI), Novosibirsk, Russian Federation.

Introduction. Approaches based on antigen presentation to cytotoxic T lymphocytes (CTLs) by dendritic cells (DCs) are actively applied in modern cancer immunotherapy. The aim of the study was to obtain $CD8^+$ T cells specific for HER2/neu (HER2) antigen and evaluate their phenotype and effector functions.
Methods. A developed protocol for obtaining HER2-specific $CD8^+$ T-lymphocytes was used, consisted of magnet-assisted transfection of monocyte-derived DCs, co-culturing of antigen-loaded DCs with autologous lymphocytes, magnet sorting of CTLs specific to HER2 epitopes and stimulation of separated CTLs by IL-2, IL-7 and IL-15. HER2-specific CTL phenotyping and identification of T memory subsets was carried out using multicolor flow cytometry. Cytotoxity assay against HER2-expressing MCF-7 cell line was performed to evaluate effector function of obtained T cells.
Results. Here we show that HER2-specific CTL populations obtained with the protocol we developed contain large percent of T stem cell-like memory cells (T_{SCM}) (about 60% of $CD8^+$ HER2-specific T cell population). Cytotoxicity analysis of HER2-specific T-cells showed significantly higher level of cytotoxic effect against cell line MCF-7, as compared with that of the fraction of activated PBMCs and that of the fraction of $CD8^+$ T cells of different specificities.
Conclusion. HER2-specific T cells obtained by the protocol we developed characterized by a high level of cytotoxicity against HER2-expressing tumor cells and which are mostly represented by T memory cells, especially T_{SCM} subset. Obtained T cells could be used for eliminating tumor cells and prevent tumor relapse after the primary tumor burden deletion in HER2-overexpressed cancer patients.

P.B4.03.16

The newly-arisen Devil Facial Tumour disease 2 (DFT2) reveals gradual loss of MHC class I as a mechanism for the emergence of a contagious cancer

*A. Caldwell[1], R. Coleby[1], M. R. Stammnitz[2], M. Tringides[1], E. P. Murchison[2], K. Skjødt[3], G. J. Thomas[1], J. Kaufman[2], T. Elliott[1], G. Woods[4], **H. Siddle**[1];*
[1]University of Southampton, Southampton, United Kingdom, [2]University of Cambridge, Cambridge, United Kingdom, [3]University of Southern Denmark, Odense, Denmark, [4]University of Tasmania, Hobart, Australia.

Devil Facial Tumour 2 (DFT2) is a recently discovered contagious cancer circulating in the Tasmanian devil (*Sarcophilus harrisii*), a species which already harbours a more widespread contagious cancer, Devil Facial Tumour 1 (DFT1). Here we show that in contrast to DFT1, DFT2 cells express major histocompatibility complex (MHC) class I molecules, demonstrating that loss of MHC is not necessary for the emergence of a contagious cancer. However, the most highly expressed MHC class I alleles in DFT2 cells are non-polymorphic or common among host devils, reducing immunogenicity in a population sharing these alleles. In parallel, MHC class I loss is emerging *in vivo*, thus DFT2 is mimicking the evolutionary trajectory of DFT1. Based on these results we propose that contagious cancers can exploit partial histocompatibility between the tumour and host, but selective pressure from the immune system will drive loss of allogeneic antigens, which could facilitate widespread transmission of DFT2. Funding: Leverhulme Trust project grant RPG-2015-103.

P.B4.03.17

TACS - traceless affinity cell selection

*H. Stadler, M. Kiene, F. Leonhardt, K. Stanar, L. Breithaupt, **M. Tietzel**;*
IBA GmbH, Göttingen, Germany.

Traceless affinity cell selection is a novel approach for positive cell isolation, which completely avoids the use of high affinity antibodies that often lead to irreversible cell binding and cause undesired stimulation of target cells. In contrast to fluorescence-activated cell sorting (FACS) and magnetic-activated cell-sorting (MACS), traceless affinity cell selection utilizes a principle, which we have termed “tag-activated cell sorting” or TACS. TACS comprises an innovative immune-affinity chromatography, whereby target cells are captured by cognate Fab fragments that are reversibly immobilized on a column matrix via an epitope tag. Specifically, low affinity Fab-fragments harboring a C-terminal Strep-tag® (Fab-Streps) are attached to Strep-Tactin®-coated cell-grade agarose, and this multimerization of Fab-Streps promotes their binding to target cells with high avidity. Hence, when a single-cell suspension is passed through a Fab-Strep/Strep-Tactin® column, target cells adhere to the affinity matrix based on the exclusive binding of the Fab-Strep to the target cell. Non-target cells are washed away efficiently. Finally, the addition of biotin triggers the elution of the target cells, and the Fab-Streps spontaneously self-dissociate from the cell surface due to the reduced affinity. This novel “Fab-TACS®” procedure delivers label-free, non-activated target cells of highly reproducible quality and purity directly from a variety of single cell suspensions, e.g. whole blood, buffy coat or rodent spleen, allowing a multitude of downstream applications. Manual Fab-TACS® gravity columns as well as fully automated Fab-TACS® cell isolation using the FABian® bench top instrument provide convenient and cost efficient options for conducting standardized TACS experiments in the laboratory.

P.B4.03.18

Effect of long-term IFN exposure on CD8 T cell functionality

***J. Mikulec**[1], M. Hofmann[2], R. Thimme[2], R. Bartenschlager[1,3];*
[1]Division of Virus-associated Carcinogenesis, German Cancer Research Center, Heidelberg, Germany, [2]Department of Medicine II, University Hospital Freiburg, Freiburg, Germany, [3]Department of Infectious Diseases, Molecular Virology, University Hospital Heidelberg, Heidelberg, Germany.

Chronic infection with the hepatitis C virus (HCV) is a major risk factor for serious liver diseases and affects around 1% of the population. A hallmark of HCV is the high propensity to establish persistence, which occurs in around 80% of infected individuals. This is achieved by suppression of both innate and adaptive immune response. Innate immunity, most notably induction of interferons (IFNs), is the first line defense limiting viral replication and determining the outcome of an infection. However, it has been shown that sustained activation of the IFN system in case of persistent *lymphocytic choriomeningitis virus* infection has detrimental impact on T cell response. Elevated IFN signature is observed in chronically HCV-infected patients displaying impaired CD8 T cell responses arguing that persistent activation of the IFN system might dampen T cell response also in chronic hepatitis C. To address this hypothesis we aim to investigate effect of long-term IFN exposure on HCV-specific CD8 T cell function. For this purpose, we take advantage of a cell-culture model, which is based on a HCV replicon-containing cell line stably transduced with the HLA-A2 gene. These cells are used as target cells to measure biological activity of HCV-specific CD8 T cells that have been cultured in the absence or presence of IFNα for extended time spans. Furthermore, we will characterize the IFN-induced changes of CD8 T cells, including their differentiation status, the expression of inhibitory molecules as well as transcription factors. By using these approaches we want to determine to what degree long-term IFN-treated T cells alter their responsiveness to HCV-infected target cells.

P.B4.03.19

Analysis of genes involved in antigen presentation to MR1T cells

***A. Vacchini**, J. Spagnuolo, L. Mori, G. De Libero;*
University Hospital and University of Basel, Basel, Switzerland.

The MHC class I-related (MR1) protein is a non-polymorphic molecule presenting bacterial metabolites to MR1-restricted MAIT cells. We have identified a novel population of MR1-restricted T cells (MR1T cells) that recognize unidentified antigenic molecules belonging to diverse classes and with small-molecular-weight. MR1T cells have polyclonal TCRs and display heterogeneous antigen specificity, recognize and kill different tumor cells in MR1-restricted manner. Activated MR1T cells release various cytokines supporting different types of immune response. Molecular mechanisms underlying the generation of self-antigens presented by MR1, their loading onto MR1 and presentation to T cells remain unknown. To identify candidate genes involved in such functions, we transduced MR1-overexpressing A375 melanoma cells with a CRISPR knock-out library (containing 123'411 sgRNAs encompassing the whole human genome) and killed them with MR1T cells, enriching for surviving A375 cells. Guide RNAs amplified from the genomes of surviving A375 cells were deep sequenced and analysed using the edgeR package.
Hits were chosen based on the strictly standardized mean difference (SSMD) calculated for the two most enriched/depleted guides for each gene target, considering an SSMD ≥3 for enriched and ≤-5 for depleted guides, compared to unkilled cells. Gene ontology analysis of significantly enriched or depleted sgRNAs revealed genes involved in different cell functions, the most abundant being those with a role in primary metabolism. Other identified genes contribute to cell adhesion, protein trafficking, metabolite transport, and RNA stability. We are currently validating selected genes that might participate in the generation/degradation of MR1T-stimulating antigens and in MR1-restricted antigen presentation.

P.B4.03.20

Functional characterization of gd T cells in human colon tumours

***W. Rodin**[1], P. Sundström[1], F. Ahlmanner[1], E. B. Lindskog[2], M. Quiding-Järbrink[1];*
[1]Dept. of Microbiology and Immunology, Göteborg, Sweden, [2]Dept. of Surgery, Göteborg, Sweden.

In colorectal cancer, tumour progression and patient outcome is affected by cytokine balance in the tumours. IFNγ, TNFα and Granzyme B production are all associated with favorable patient outcome, while high IL-17 expression is associated with accelerated tumour progression. However, the knowledge of regulation and activation of intraepithelial T cells in colon cancer is still limited. The aim of this study was to characterize intestinal intraepithelial T cells in colon tumours and unaffected tissue as well as to determine their capacity to produce cytokines affecting tumour progression, using flow cytometry. We show that the frequencies of Vδ1$^+$ γδ T cells and CD8$^+$ T cells are reduced in the epithelium of colon tumors compared to unaffected tissue. Intraepithelial T cells in both tissues express markers associated with an activated memory phenotype and moderate levels of PD-1. On a per cell basis the tumour epithelium contains higher frequencies of γδ and αβ T cells producing IFN-γ and TNFα compared to the unaffected tissue. Likewise, frequencies of IL-17 producing cells are also higher in IELs from tumours, but much lower than the T_H1 - associated cytokines. Granzyme B production was observed in γδ and αβ T cells both before and after activation and is also expressed at higher levels in tumour IELs compared to IELs from unaffected tissue. Taken together, this study indicates that IELs actively contribute to the cytokine balance in colon tumours with a T_H1 – dominated profile, which may influence tumour progression and patient outcome.

P.B4.03.21

Depletion of intratumoural regulatory T cells by the anti-ICOS antibody KY1044 in combination with immune checkpoint blockade enhances the anti-tumour response in pre-clinical models

*R. C. Sainson, A. Thotakura, N. Praveen, **M. Kosmac**, G. Borhis, J. Carvalho, T. Okell, R. Rowlands, H. Ali, H. Craig, V. Wong, Q. Liang, V. Germaschewski, M. McCourt;*
Kymab, Cambridge, United Kingdom.

Inducible T-cell costimulator (ICOS/CD278) is a CD28 superfamily member that is induced when T cells are activated. Although ICOS expression levels vary in different T cell subtypes and in different tissues, in preclinical mouse tumour models, we have observed that nearly all T regulatory cells (TRegs; CD4+/FOXP3+) express ICOS on their surface and that the expression level of ICOS on TRegs is significantly higher than that on effector T cells (TEffs). In addition, ICOS expression on TRegs in the tumour microenvironment is higher than that of TRegs in the blood or spleen, which makes it a strong candidate for cancer immunotherapy via TReg depletion. By immunizing Kymice™, in which the endogenous *Icos* gene has been knocked out, we have identified a novel, cross-reactive, fully human antibody called KY1044 that selectively binds to dimeric ICOS (Fc fusion protein) with an affinity of less than 2nM. Using in vitro and in vivo approaches we demonstrate that KY1044 has dual mechanisms of action: (1) it promotes the preferential depletion of intratumoural ICOS^{high} TRegs resulting in an increase in the TEff:TReg ratio in the TME; and (2) it co-stimulates ICOS^{low} TEff cells. Notably, our pharmacodynamic studies demonstrated long-term durable depletion of TRegs and a significant increase in the TEff:TReg ratio in response to KY1044 treatment, an effect that is crucially dependent on CD8+ but not CD4+ T cells. In summary, our data demonstrate that targeting ICOS with KY1044 is a valid approach for modulating the immune system and for inducing a strong anti-tumour response in multiple indications.

P.C1.01 Maintenance and local regulation of tissue specific immunity - Part 1

P.C1.01.01

Negative Checkpoint Receptor (NCR) expression in Graves' disease thyroid tissue

***D. Álvarez-Sierra**[1], A. Marín-Sánchez[2], P. Ruiz-Blázquez[1], C. Iglesias-Felip[3], P. Nuciforo[4], Ó. González-López[5], A. Casteras[6], G. Obiols[6], R. Pujol-Borrell[2];*
[1]Vall d'Hebron Research Institute, Barcelona, Spain, [2]Department of Immunology, Hospital Universitari Vall d'Hebron, Barcelona, Spain, [3]Department of Pathology, Hospital Universitari Vall d'Hebron, Barcelona, Spain, [4]Vall d'Hebron Institute of Oncology, Barcelona, Spain, [5]Department of Surgery, Hospital Universitari Vall d'Hebron, Barcelona, Spain, [6]Department of Endocrinology, Hospital Universitari Vall d'Hebron, Barcelona, Spain.

It has been reported that autoimmune thyroiditis occurs frequently after cancer immunotherapy with anti-PD-1/PD-L1.
Since PD-L1 can be induced by IFN-γ and in Graves' Disease (GD) thyroid there is a clear IFN signature, we predicted that thyrocytes, even in focal thyroiditis, would express PD-L1. Anti-PD-L1 treatment would then exacerbate focal thyroiditis and lead to clinical thyroid autoimmunity. The results here presented suggest another scenario.
Cryostat sections of GD, multinodular goitre and control lymphoid organs were stained by multicolour immunofluorescence for PD-1, PD-L1, as well as for HLAI and HLAII, Cytokeratin-18, TPO and phenotypic markers. Against our prediction, in most GD glands, PD-L1 was undetectable in thyrocytes or present at very low level, certainly much less conspicuous than HLAII. Importantly, PD1 was highly expressed by the infiltrating T cells.
Primary thyroid and thyroid-derived cell lines cultures were supplemented with increasing doses of IFNγ and stained at 24h-48h to assess HLA-I/II, PD-L1 and PD-L2 expression induction. PD-L1 and PD-L2 expression was readily induced by IFNgamma in thyrocytes cultures in a time and dose dependent manner, as assessed by flow cytometry and qPCR, demonstrating the capability of thyrocytes to express PD-1 ligands.
Overall these results suggest that the autoimmune thyroiditis linked to anti-PD-1 treatment is not due a local effect of anti-PD1/PD-L1 drugs blocking the interaction between PD-1 positive T cells and thyrocytes expressing PD-L1 o PD-L2. The factor(s) inhibiting thyrocytes PDL1 induction in vivo remain to be elucidated.

P.C1.01.02

Sexual dimorphism in mechanisms controlling development of CD4+ T cell response in collagen-induced arthritis

***B. Bufan**[1], N. Arsenović-Ranin[1], M. Dimitrijević[2], M. Nacka-Aleksić[3], D. Kosec[4], I. Pilipovic[4], M. Stojanović[3], G. Leposavić[3];*
[1]Department of Microbiology and Immunology, University of Belgrade Faculty of Pharmacy, Belgrade, Serbia, [2]Department of Immunology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia, [3]Department of Physiology, University of Belgrade Faculty of Pharmacy, Belgrade, Serbia, [4]Immunology Research Centre "Branislav Janković", Institute of Virology, Vaccines and Sera ''Torlak'', Belgrade, Serbia.

Introduction: Considering sex bias in rheumatoid arthritis prevalence, influence of biological sex on the disease development in Dark Agouti rat collagen II (CII)-induced arthritis (CIA) model of the human disease was examined. Methods: Sex bias in CD4+ T cell responses in inguinal (draining the site of immunization in preclinical CIA) and popliteal (draining inflamed joints at the peak of CIA) lymph nodes (LNs) and mechanisms controlling their development were examined using flow cytometry and/or ELISA/qRT-PCR. Results: In both inguinal and popliteal LNs greater number of CD4+CD25+Foxp3- cells, presumably activated effector T cells, was found in females compared with males, and they exhibited greater CII-specific proliferation. Consistently, more IL-17+, IFN-γ+ and IL-17+IFN-γ+ T cells were retrieved from both inguinal and popliteal female rat LNs. Moreover, more GM-CSF+ and IL-17+IFN-γ+GM-CSF+ T cells were retrieved from female compared with male rat popliteal LNs. On the other hand, lower frequency of PD-1+ cells among CD4+CD25+Foxp3+ regulatory T cells (Tregs) from female popliteal and inguinal LNs suggested lower suppressive capacity of their Tregs. Additionally, from female rat popliteal LNs fewer Tregs were recovered. Furthermore, the number of regulatory LN B10 cells was lower in females. Moreover, compared with males, in females was shifted LN INF-γ+/IL-4+ T-cell ratio towards the former, and accordingly serum CII-specific IgG2a/IgG1 antibody ratio was shifted towards pathogenic IgG2a antibodies. Conclusion: The study suggests that a less efficient control of (auto)immune Th1/Th17 cell responses during CIA development contributes to sex bias in the susceptibility to CIA. (Grant 175050, MESTD of Serbia).

P.C1.01.03

ΔdblGATA mice are resistant to experimental autoimmune encephalomyelitis

***B. Ciric**, D. Hwang, A. Rostami;*
Thomas Jefferson University, Philadelphia, United States.

GATA-binding factor 1 (GATA-1) is a transcription factor expressed in certain hematopoietic cells, governing their development and function. GATA-1 is expressed in myeloid cells but not in lymphoid cells, such as T cells. Since knockout of GATA-1 is embryonic lethal, mouse lines lacking individual enhancers to the GATA-1 gene have been generated. Lack of a particular enhancer selectively reduces, or precludes GATA-1 expression in some cell types, while in others its expression remains normal. We use mice lacking dblGATA enhancer (ΔdblGATA mice), which are devoid of eosinophils. We found that ΔdblGATA mice are resistant to both direct and adoptive EAE. ΔdblGATA mice develop weaker myelin-specific Th responses upon immunization for EAE induction, indicating a defect in priming immune responses. The resistance of ΔdblGATA mice to adoptive EAE indicates a deficiency in effector mechanisms of CNS inflammation. We have ruled out lack of eosinophils as the reason for EAE resistance of ΔdblGATA mice by demonstrating that another mouse strain (PHIL), which also lacks eosinophils, develops normal EAE. These findings show that ΔdblGATA mice have defects in both the priming and effector phases of disease. Additionally, immunized ΔdblGATA mice had fewer inflammatory monocytes and myeloid dendritic cells in blood, draining lymph nodes and the CNS. Our findings thus far suggest that an unknown defect in monocytes/dendritic cells causes resistance to EAE in ΔdblGATA mice.

P.C1.01.04

MAF expression in regulatory and conventional T cells controls gut homeostasis

***C. Imbratta**[1], M. Leblond[1], D. Velin[2], H. Bouzourène[3], D. Speiser[4], G. Verdeil[1];*
[1]Clinical Tumor Biology & Immunotherapy Group, Department of Fundamental Oncology, University of Lausanne, Epalinges, Switzerland, [2]Service of Gastroenterology and Hepatology, CHUV, Lausanne, Switzerland, [3]Unilabs, Epalinges, Switzerland, [4]Clinical Tumor Biology & Immunotherapy Group, Department of Fundamental Oncology, University of Lausanne, CHUV, Epalinges, Switzerland.

The maintenance of homeostasis in the gut is a major challenge for the immune system. In an attempt to characterize the role of the transcription factor MAF in T cells, we demonstrated that it is a key regulator of homeostasis in the gut. Mouse knocked out for *maf* in all T cells developed a spontaneous late-onset colitis, correlating with the disappearance of FOXP3+ RORγt+ T cells from the colon that normally express high level of MAF and is the major source of IL-10. We demonstrated that classical T regulatory cells inactivated for *maf* are defective in controlling colitis development in a co-transfer model and are not able to cope for the defect of RORγt+ Treg in the colon. Furthermore we show that Treg specific *maf*-KO are not sufficient to induce colitis and that the deletion of *maf* in conventional T cells is also necessary for the development of the disease.

P.C1.01.05

TCR repertoire of PD-1+ and CD137+ T-cells from synovial fluid of patients with spondyloarthropathies

***E. A. Komech**[1,2], A. D. Koltakova[3], T. V. Korotaeva[3], E. Y. Loginova[3], E. I. Shmidt[4], Y. B. Lebedev[1,2], I. V. Zvyagin[1,2];*
[1]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russian Federation, [2]Pirogov Russian National Research Medical University, Moscow, Russian Federation, [3]Nasonova Research Institute of Rheumatology, Moscow, Russian Federation, [4]City Clinical Hospital #1, Pirogov Russian National Research Medical University, Moscow, Russian Federation.

Identification of auto-reactive T-cell clones could provide a useful diagnostic tool and treatment target for autoimmune diseases. To recognize clones, that are directly involved in pathologic process, we focused on TCR repertoire of activated PD-1+ and CD137+ T-cells from synovial fluid (SF) samples of patients with ankylosing spondylitis (AS) and psoriatic arthritis.
Using quantitative molecular-barcoded 5'-RACE, we performed TCRβ repertoire profiling of activated T-cells: CD3+PD-1med/hi, CD3+CD4+CD137+ and CD3+CD8+CD137+ cells, that were FACS-sorted from SF samples of patients.
From 57 to 83.7% of T-cells from SF were PD-1+, and CD137+ T-cells comprised on average 7.5% from either CD4+ and CD8+ T-cells. Clonal diversity of PD-1+ T-cells was not different from PD-1- cells. Most of CD8+CD137+ T-cells were found in PD-1+ subset of the same donor. Only a few clonotypes of activated fractions were shared between donors, including one clonotype from recently identified AS-associated TCRβ motif. In repertoire of the activated subsets we observed several low abundant clonotypes with known specificity for CMV, EBV, Influenza and melanA protein.
Our results show that the majority T-cells from synovial fluid of patients with spondyloarthropathies are activated. T-cell clonotypes of activated fractions are rather unique for each donor. Presence of clonotypes matching AS-associated TCR motif in activated T-cell subsets of synovial fluid of patients with different spondyloarthropathies supports their role in pathogenesis of the diseases.
Funding: RSF grant No 17-75-10220.

P.C1.01.06

IL-37 increases inflammation in murine immune-mediated liver diseases

***C. Lin**, Y. Chuang;*
Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, Taipei, Taiwan.

IL-37 is an emerging IL-1 family cytokine with anti-inflammatory effects on innate and adaptive immunity that shows benefit on several autoimmune diseases. Primary biliary cholangitis (PBC) is a chronic autoimmune disease that occurred in the liver with innate and adaptive immune cell infiltration in the portal triad, followed by fibrosis and cirrhosis. To investigate whether IL-37 can alleviate PBC, we used adeno-associated virus as a vector to express IL-37 in the liver of PBC mice. We found that the features of PBC, including hepatic infiltrating lymphocytes, serum autoantibody, hepatic inflammatory cytokine expression and the liver histopathology were no differences between AAV-hIL-37 or AAV-mock administered mice. However, polymorphic nuclear cells (PMNs) in liver were increased in AAV-hIL-37 injected PBC mice. To further extend whether IL-37 recruit PMN in the immune mediated liver disease, we used Concanavalin A (Con A)-induced hepatitis mouse model. Compared to AAV-mock group, Con A injected mice administered with AAV-hIL-37 had significant increases in not only PMNs but also NK, NKT, and dendritic cells in the liver. In addition, serum IFN-γ was significantly increased in AAV-hIL-37 injected Con A mice. In conclusion, IL-37 induces PMN infiltration in autoimmune cholangitis and Con A induced hepatitis and shows pro-inflammatory effects in Con A induced hepatitis.

P.C1.01.07

Deletion of the mineralocorticoid receptor in myeloid cells leads to reduced EAE severity

***F. Lühder**[1], E. Montes-Cobos[2], N. Schweingruber[1], A. Flügel[1], H. Reichardt[2];*
[1]Institute for Multiple Sclerosis Research, Göttingen, Germany, [2]Institute for Cellular and Molecular Immunology, Göttingen, Germany.

Myeloid cells play important roles in different cruial steps during the pathogenesis of multiple sclerosis and its animal model experimental autoimmune encephalomyelitis (EAE). Peripheral myeloid cells act as APCs for the initial activation of autoreactive T cells in peripheral lymphoid organs, whereas local myeloid cells are presumably responsible for their re-activation within the CNS. Myeloid cells such as monocytes and macrophages, but also local CNS-resident ones such as microglia, can differentiate towards distinct phenotypes, M1 myeloid cells being more related towards inflammation and autoimmunity and M2 myeloid cells being more related to tissue repair and anti-inflammatory processes. We generated a mouse model specifically lacking the mineralocorticod receptor (MR) in myeloid cells (MR^{LysM} mice). In these mice, the phenotype of bone marrow-derived and peritoneal macrophages was modulated towards M2 including differential phagocytic and migratory properties and NO metabolism. Furthermore, these mice presented with diminished clinical symptoms and ameliorated histological hallmarks of neuroinflammation after EAE induction by MOG_{35-55} and CFA. T cells in peripheral lymphoid organs of these mice produced less pro-inflammatory cytokines while their proliferation and the abundance of regulatory T cells were unaltered. In the CNS, the numbers of inflammatory monocytes and reactive microglia in MR^{LysM} mice were significantly lower and they also adopted an M2-polarized phenotype based on their gene expression profile, presumably explaining the ameliorated neuroinflammation. Thus, the lack of the MR in myeloid cells impacts CNS autoimmunity by either direct effects and/or modulation of the adaptive immune response.

P.C1.01.08

Tissular immune dysregulation in human autoimmune thyroiditis

***A. Mohr**[1], C. Trésallet[2], N. Monot[1], A. Bauvois[1], D. Abiven[3], L. Leenhardt[4], G. Gorochov[3], M. Miyara[3];*
[1]Sorbonne Université, Inserm, Centre d'immunologie et des maladies infectieuses – Paris (CIMI-PARIS), Paris, France, [2]Sorbonne Université, Service de Chirurgie Générale, Viscérale et endocrinienne, AP-HP Hôpital Pitié-Salpêtrière, Paris, France, [3]Sorbonne Université, Inserm, Centre d'immunologie et des maladies infectieuses – Paris (CIMI-PARIS), AP-HP Hôpital Pitié-Salpêtrière, Paris, France, [4]Sorbonne Université, Service d'endocrinologie, AP-HP Hôpital Pitié-Salpêtrière, Paris, France.

Introduction: Peripheral abnormalities in Th17 and regulatory T cells have been observed in autoimmune thyroid diseases (AITDs) that include Hashimoto's thyroiditis (HT) and Grave's disease (GD). However, it is still unclear whether such abnormalities occur in the thyroid tissue.
Materials and Methods: PBMCs and thyroid tissue infiltrating immune cells from 18 patients with HT, 10 patients with GD and 30 control donors (CD) have been analyzed by flow cytometry in order to study T cells and ILC subsets.

Results: We did not observe the previously reported increase of peripheral TH17 cells or the decrease of circulating Treg cells in HT and GD patients. $CXCR5^{+}PD1^{hi}$ TFh cells and LTi-like ILC3 were significantly increased among immune cells infiltrating thyroid from patients with both HT and GD. No modification in $FOXP3^{hi}$ effector Treg (Fr. II) cells was observed in AITDs tissue when compared to controls, while IL-2 producing $FOXP3^{low}CD4^{+}$ T (Fr. III) cells were increased. In addition, the presence of $CXCR5^{+}FOXP3^{low}CD4^{+}$ TFR-like cells with low levels of CTLA-4 was also suggestive of defective local immune dysregulation in AITDs.
Conclusion: AITDs are characterized by the occurrence of tissue immune dysregulation as the presence of TFH and ILC3, two populations involved in secondary and tertiary lymphoid organ development, and IL-2 producing $FOXP3^{low}CD4^{+}$ T (Fr. III) and $CXCR5^{+}FOXP3^{low}CD4^{+}CTLA\text{-}4^{+}$ TFR-like predominating in patients. Those abnormalities may promote the local production of autoantibodies.

P.C1.01.09

Immunological biomarkers of autoimmune liver disease in patients without clinical symptomatology

*A. Navas[1], M. López[2], **J. Molina**[3,1], A. Jurado[3,1];*
[1]Maimonides Biomedical Research Institute of Cordoba (IMIBIC)/ Reina Sofia University Hospital/ University of Cordoba, Cordoba, Spain, [2]Department of Clinical Analyses, Reina Sofia University Hospital, Cordoba, Spain, [3]Department of Allergy and Immunology, Reina Sofia University Hospital, Cordoba, Spain.

Primary biliary cirrhosis (PBC) is an autoimmune disease which results in a slow and progressive destruction of the liver small bile-ducts. PBC is more common in medium-age women and its diagnosis is usually hazard-made according to clinical symptoms, such as tiredness, itching and an increased level of liver-enzymes routinely examined. Autoantibodies against gp-210 and sp-100 are found in association with PBC, together with anti-mitochondrial antibodies. The aim of this study was to analyze demographics and clinical characteristics of serum samples screened for autoantibodies with positivity to anti-gp-210 and sp-100 antibodies.
In this study 75 patients were included. Serum sp-100 and/or gp-210 autoimmune biomarkers were detected by immune-blot according to the immunofluorescence result of autoantibodies on Hep-2 cells. Levels of transaminases were measured by chemiluminescence. The diagnostic suspicion was registered.
The 92% of patients were women. Medium-age of all patients was 59.6±13.5. Anti-sp100 and anti-gp210 were positive in 49 (65.3%) and 20 (26.7%) patients, respectively.
In 5 patients (6.7%) both antibodies were detected. Medium level of liver-enzymes was 43.9±42.3 for aspartate-aminotransferase, 43.07±50.1 for alanine-aminotransferase, 177.1±248.9 for gamma-glutamyl-transferase and 153.9±180.2 for alkaline-phosphatase. The majority of the samples came from the Rheumatology Department (29.3%), followed by those from the Digestive Department (21.3%). Only 17 (22.7%) samples belonged to patients with the clinical suspicion of PBC.
Samples from medium-age women routinely examined with an increased level of transaminases may exhibit a positivity to PBC autoantibody biomarkers. Interestingly, the majority of them are not clinically suspicious of suffering it.

P.C1.01.10

Assessment of the DFS-70 (anti-dense fine speckled 70 antibodies) specificity by CIA (Chemiluminescent immunoassay) and blot in 96 serum samples with the ICAP AC-2 immunofluorescence pattern

***D. Monzón Casado**[1], M. De Juan[1], M. Imaz Ocharan[2], M. Rey Rey[1], L. Aragón Irusquieta[1], Á. Prada Iñurrategui[1];*
[1]Osakidetza, San Sebastián, Spain, [2]Osakidetza, Bilbao, Spain.

Introduction: The nuclear dense fine speckled (ICAP AC-2) pattern in Hep-2 are observed in autoimmunity laboratories. These autoantibodies have been detected with low frequency in systemic autoimmune rheumatic diseases (SARD), in healthy individuals and in patients with other diseases.
Objective: We selected serum from 96 patients based on an immunofluorescence screening of ANA (Tittle ≥ 1/160 and ICAP AC-2 pattern). We investigated anti DFS-70 antibodies using chemiluminescence (CIA) immunoassay and blotting..
Material and Methods.
We analized 96 sera by IFA screening (Euroimmun). DFS-70: CIA (Quanta Flash Werfen) and Blot (Euroimmun). ANAs specificities: Blot (Euroimmun).
dsDNA antibodies: Enzyme immunoassay (Thermofisher) and IFA (*Crithidia luciliae*)..
Results: 1.- DFS antibodies.
From the 96 ICAP-AC2: 12/96 were positive by blot and 6/96 by CIA.
Using a binomial distribution with an uninformative beta prior, we estimated that the probability the Blot detects more positive results than the CIA is 0.9305.
2.- ANAs specificity
Three patients DFS-70 positive by blot were positive for RNP, Histones and Cenp-B.
An ICAP AC-2 positive DFS-70 negative cohort (n=14) were positive for histones (2p) nucleosomes (1p), Scl-70 (2p), Ro (4p), and others.
The ICAP AC-2 positive cohort determined by IFA (n=79) resulted negative for anti DFS-70 and other nuclear specificities.
3.- Anti-dsDNA.
All the patients were negative. .
Conclusions: 1.- DFS-70 monospecific antibodies are present in a low fraction of ICAP-AC2.
2.- The Blot has more positive results than the CIA.
3.- Besides DFS-70, other unknown specificities might show IFA AC-2 pattern.

P.C1.01.11

The role of the tetraspanin CD82 in rheumatoid arthritis synovial fibroblast migration and inflammation

***E. Neumann**[1], M. Schwarz[1], R. Hasseli[1], M. Hülser[1], S. Classen[2], M. Sauerbier[3], S. Rehart[4], U. Müller-Ladner[1];*
[1]Dept Rheumatology and Clinical Immunology, Campus Kerckhoff, Justus-Liebig-University Giessen, Bad Nauheim, Germany, [2]Divis. Vascular Surgery, Kerckhoff-Klinik GmbH, Bad Nauheim, Germany, [3]Dept Plastic, Hand and Reconstructive Surgery, BGU, Frankfurt, Germany, [4]Dept of Orthopaedics and Trauma Surgery, Agaplesion Markus Hospital, Frankfurt, Germany.

Introduction: Tetraspanins are membrane adaptors altering cell-cell fusion, receptor-mediated signal transduction and cell motility via interaction with membrane proteins including adhesion molecules such as integrins. CD82 is expressed in several malignant cells and a well described tumor metastasis suppressor. Rheumatoid arthritis (RA) is based on synovial inflammation and joint destruction driven in part by activated synovial fibroblasts (SF) with an increased migratory potential. CD82 is upregulated in RASF vs. osteoarthritis (OA) SF and within RA vs. OA synovial tissue. Therefore, the role of CD82 in RASF was evaluated.
Methods: Double-immunofluorescence for CD82 and integrins, proinflammatory stimuli induction of CD82, lentiviral CD82-overexpression, siRNA-mediated knockdown, RASF migration and attachment assays towards plastic/matrigel, RASF-binding assays to endothelial cells (EC), and evaluation of CD82 expression during long-term invasion in the SCID-mouse-model were performed.
Results: CD82 was significantly induced by proinflammatory stimuli (e.g. RASF: TNFα 9.55-fold; IL-1β 12.54-fold). In RA-synovium, CD82 was expressed in RASF close to vessels, lining layer, sites of cartilage invasion, and colocalized with integrins involved in tumor metastasis suppression and RASF attachment. CD82 overexpression reduced RASF migration (e.g. 46.4±15.7 vs. RFP, p=0.0004), cell-matrix (e.g. 27.8% vs. leRFP (100%), p=0.0314) and RASF-EC adhesion. CD82 knockdown increased RASF migration (CD82 174.5±70.0, NTP 74.5±70.1, p=0.044) and matrix adhesion (147.7% vs. NTP, p=0.0166) whereas RASF-EC-interaction was reduced (60.7% vs. NTP, p=0.0015). In SCID mice, the presence of CD82 on cartilage-invading RASF was confirmed.
Conclusion: CD82 contributes to RASF migration to sites of inflammation and tissue damage but also to perpetuation of RASF to articular matrix.

P.C1.01.12

Association of HLA-A alleles with vitiligo in Turkish people

*Y. Hayran, G. O. Ergen, **F. Ozmen**;*
Department of Basic Oncology, Cancer Institute, Hacettepe University, Ankara, Turkey.

Background and Aim: Vitiligo is an autoimmune disease of the skin that results in the destruction of melanocytes and the clinical appearance of white spots.The aim of this study was to investigate the association of HLA-A alleles with the extent and course of disease in patients with vitiligo. **Patients and Methods:** The study included 71 patients with vitiligo and the control group included 100 unrelated healthy donors. HLA-A typing was performed by using PCR-SSO method at low resolution level. **Results :**71(40F) patients with Vitiligo included in our study which consist of 46(64.8%) vitiligo vulgaris.Extensiveness of disease was 7.75(0.1-45)% and commence age(mean) of the disease was 26.5 years. 22.5% (n=16) male patients had positive family history. HLA-A*02 allelle frequency was found to be significantly higher in vitiligo patients than control group *(p=0.023).* Each allele increase the risk of vitiligo by two fold *(CI=1.19-3.34)*. HLA-A*02 allele frequency was similar between the sub-types of vitiligo. HLA-A*02 allele frequency was also found to significantly correlated with commence age of the disease *(p=0,026; r=0,266)*. When compared to the patient, HLA-A*11 was found to significantly higher in controls *(p=0.008)*. HLA-A*11 was found to be preventive for vitiligo disease and each allele was preventive as 3.4 folds(CI=1.31-8.88). There were no statistical correlation between HLA-A*02 allele frequency and extensiveness of the disease (VASI), degree of pigmentation, disease activity(VADI), associated auto-immune disease and the presence of vitiligo in the family. **Conclusion:** This results suggest that HLA-A*02 allele might be positively associated with vitiligo in Turkish people.

P.C1.01.13

Molecular mechanisms implicated in the pathogenesis of multiple sclerosis

I. Panagoulias[1], *I. Aggeletopoulou*[2], *S. Anastasopoulou*[2], *E. Kourepini*[3], *E. Pappou*[2], *T. Georgakopoulos*[2], *V. Panoutsakopoulou*[3], *A. Mouzaki*[2];
[1]*Medical School, University of Patras, Patras, Greece,* [2]*Medical School, Patras, Greece,* [3]*Biomedical Research Foundation of the Academy of Athens, Athens, Greece.*

In MS, pathogenic Th-cells (mainly Th1 and Th17) recognize myelin antigens and contribute to the damage to the CNS. An important unresolved issue of MS pathogenesis is at which stage of Th-cell differentiation errors occur, at the molecular level, that result in the development of autoreactive Th-cells. We previously showed that in healthy individuals the IL-2 gene is repressed in naive Th-cells by the transcription factor Ets-2, that binds to ARRE-2 element (distal NFAT binding site) of the proximal IL-2 promoter, pointing to Ets-2 as a crucial factor influencing early events of Th-cell differentiation. Importantly, we also demonstrated that Ets-2 suppresses the expression of other genes with Ets-2 binding sites, including cytokines and HIV-1. Our results from 10 patients with remitting-relapsing MS (4M/6F, age: 24-38y, disease duration: 2-7 y, EDSS: 1-2.5), and 10 age/sex-matched healthy controls, show significantly reduced mRNA and protein synthesis of Ets-2 in naive Th-cells from MS patients, lack of Ets-2 binding to the ARRE-2 of the IL-2 promoter in vitro and in vivo, and significantly higher constitutive expression levels of cytokines in the patients' Th-cells (IL-2, IL-17 in naive Th-cells and TNF-α, IFN-γ in memory Th-cells). We also found significantly higher ets-2 expression in undifferentiated murine Th-cells compared to in vitro differentiated Th-cell populations, and elevated ets-2 expression in Th-cells from mice resistant to EAE. We suggest that in MS patients low-level synthesis and dysfunction of Ets-2 in Th-cells are responsible for downstream aberrant Th-cell differentiation resulting in the creation of pathological Th1 and Th17 cells.

P.C1.01.14

Dominant protection from HLA-linked autoimmunity by antigen specific regulatory T cells

J. D. Ooi[1], ***J. Petersen***[2,3], *Y. H. Tan*[2], *M. Huynh*[1], *Z. Willet*[1], *S. H. Ramarathinam*[2], *P. J. Eggenhuizen*[1], *K. L. Loh*[2], *K. A. Watson*[4], *P. Gan*[1], *M. Alikhan*[1], *N. L. Dudeck*[2], *A. Handel*[5], *B. G. Hudson*[6], *L. Fugger*[7], *D. A. Power*[8], *S. G. Holt*[9], *P. Coates*[10], *J. W. Gregersen*[11], *A. W. Purcell*[2], *S. R. Holdsworth*[12,13], *N. L. La Gruta*[2,4], *H. H. Reid*[2], *J. Rossjohn*[2,14,15], *A. Kitching*[12];
[1]*Centre for Inflammatory Diseases, Monash Medical Centre, Clayton, Australia,* [2]*Biomedicine Discovery Institute, Monash University, Clayton, Australia,* [3]*ARC Centre of Excellence in Advanced Molecular Imaging, Monash University, Clayton, Australia,* [4]*Department of Microbiology and Immunology, Peter Doherty Institute, Melbourne, Australia,* [5]*Department of Epidemiology and Biostatistics,, University of Georgia, Athens, United States,* [6]*Department of Medicine, Vanderbilt University Medical Center, Nashville, United States,* [7]*Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom,* [8]*Department of Nephrology, Austin Health, Heidelberg, VIC, Australia,* [9]*Department of Medicine, University of Melbourne, Melbourne, Australia,* [10]*Adelaide Renal and Transplantation Service, Royal Adelaide Hospital, Adelaide, Australia,* [11]*Department of Medicine, Viborg Regional Hospital, Viborg, Denmark,* [12]*Department of Nephrology, Monash Health, Clayton, Australia,* [13]*Department of Nephrology, The Royal Melbourne Hospital, Parkville, Australia,* [14]*ARC Centre of Excellence in Advanced Molecular Imaging, Monash University, Clayton, Austria,* [15]*Institute of Infection and Immunity, Cardiff University School of Medicine, Cardiff, United Kingdom.*

Susceptibility and protection against autoimmune diseases including Rheumatoid Arthritis, Multiple Sclerosis, Type I Diabetes and Goodpasture disease (GPD) is associated with particular human leukocyte antigen (HLA) alleles. T cell responses against HLA self-epitopes are considered key drivers of autoimmunity, however the molecular mechanisms that link specific HLA alleles to the initiation or suppression of autoimmunity are presently unclear. Typically, the diversity of T cell self-epitopes and the breadth of HLA associations preclude a definititive analysis of the underlying molecular mechanisms. GPD is an HLA-linked autoimmune renal disorder, where the immune response is dominated by a single CD4+ T-cell self-epitope (α3) derived from type IV collagen. While HLA-DR15 confers a markedly increased disease risk, the HLA-DR1 allele is dominantly protective. HLA-DR15 and HLA-DR1 exhibit distinct peptide repertoires and binding preferences, and the α3 epitope is presented in different binding registers. The difference in α3 presentation is associated with functionally distinct T cell repertoires: HLA-DR15-α3 tetramer+ T cells in HLA-DR15 transgenic mice exhibit a conventional T-cell phenotype (Tconv) that secretes pro-inflammatory cytokines. In contrast, HLA-DR1-α3 tetramer+ T cells in HLA-DR1 and HLA-DR15/DR1 transgenic mice are predominantly CD4+Foxp3+ regulatory T cells (Treg cells) expressing tolerogenic cytokines. HLA-DR1-induced Treg cells confer resistance to disease in HLA-DR15/DR1 transgenic mice. Our results provide a mechanistic basis for the dominantly protective effect of HLA in autoimmune disease, whereby HLA polymorphism shapes the relative abundance of self-epitope specific Treg cells that leads to protection or causation of autoimmunity.

P.C1.01.15

Staphylococcus aureus **induced Th17 cell accumulation aggravates renal autoimmune disease**

D. Reimers[1], *P. Bartsch*[2], *U. Panzer*[2], *S. Klinge*[1], *H. Rohde*[3], *C. Krebs*[2], *H. Mittrücker*[1];
[1]*Institute of Immunology - University Medical Center Hamburg-Eppendorf, Hamburg, Germany,* [2]*III. Medical Clinic and Polyclinic - University Medical Center Hamburg-Eppendorf, Hamburg, Germany,* [3]*Institute of Medical Microbiology, Virology and Hygiene - University Medical Center Hamburg-Eppendorf, Hamburg, Germany.*

CD4$^+$ Th17 cells are defined by their production of IL-17 cytokines and the expression of the transcription factor RORγt. Th17 cells are responsible for the clearance of extracellular pathogens. However, these cells also participate in autoimmune diseases such as crescentic glomerulonephritis (cGN). In our project we infect mice with *Staphylococcus aureus* and characterize the systemic and renal T cell response to infection. In addition, we induce cGN and determine the impact of prior *S. aureus* infection on the course and severity of renal disease.
S. aureus injection led to infection of the kidneys accompanied by a massive renal accumulation of T cells at day 10 post infection. Characterization of renal T cells revealed dominant populations of RORγt-positive IL-17A-secreting Th17 cells and γδ T cells. Both T cell populations were rather stable, even 10 weeks after infection increased numbers of renal Th17 and γδ T cells were still detectable. Furthermore, renal RORγt-positive T cells showed a tissue resident phenotype, defined by CD69 expression. 10 weeks after infection, cGN was induced by injection of sheep IgG binding to the glomerular basement membrane. As compared to nephritic control mice, mice with preceding *S. aureus* infection exhibited augmented renal injury with severe loss of renal function, enhanced glomerular crescents formation and massive tubulointerstitial damage.
In conclusion, our results demonstrate that renal infection and accumulation of Th17 cells correlates with an aggravated autoimmune disease. Currently, we aim to identify the underlying mechanisms.

P.C1.01.16

Systemic lupus and reproduction, lessons from the animal models

G. Boneva[1], *V. Kostadinova*[1], *J. Prechl*[2], *N. Mihaylova*[1], *S. Delimitreva*[3], ***A. I. Tchorbanov***[1];
[1]*Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria,* [2]*Immunology Research Group, Hungarian Academy of Sciences, Budapest, Hungary,* [3]*Department of Biology, Medical University Sofia, Sofia, Bulgaria.*

Introduction: Systemic Lupus Erythematosus (SLE) is a polygenic autoimmune disorder involving multiple organs that can influence female fertility. Pristane-induced mouse model of SLE is very suitable to study female fertility compared to the healthy animals with the same background. Materials and Methods: Lupus-like symptoms were induced through intraperitoneal injection of hydrocarbon oil pristane in non-autoimmune Balb/c mice. Flow cytometry was used for the detection of CD25/CD69 activation markers and apoptosis assay. The levels of cytokines, autoantibodies in the sera and the number of autoantibody-producing plasmocytes were quantified by ELISA, ELISpot and protein array. Results: A single i.p. injection of pristane leads to the development of the typical SLE symptoms such as production of different autoantibodies accompanied by massive glomerular depositions of IgG-containing immune complexes in the kidneys, and proteinuria. After hormonal ovarian stimulation, ovulated oocytes were derived from oviducts. Chromatin, tubulin and actin structures were detected by Hoechst 33258, FITC-labeled alpha-tubulin antibody and rhodamine-labeled phalloidin, respectively. The total number of obtained metaphase oocytes from lupus mice was significantly lower compared to healthy controls. The maturation rate, i.e. the proportion of eggs reaching metaphase II, was also lower for Lupus mice compared to control animals. For each oocyte, four characteristics were described - spindle morphology, actin cap, chromosomal condensation and alignment. Many specific abnormalities in the lupus group were found. Conclusions: Pristane-induced mouse model of lupus exhibited numerous impairments of the reproductive system which may result due to disease activity, autoantibodies or damage in molecular mechanisms through the process of reproduction.

P.C1.01.17

Enrichment of pioneer CD4+ T cells andup regulation of CX3CR1 in Experimental Autoimmune Uveoretinitis

A. Ward, *O. H. Bell, D. A. Copland, A. D. Dick, L. B. Nicholson;*
University of Bristol, Bristol, United Kingdom.

Non-infectious uveitis is a T cell mediated intraocular inflammatory disease leading to severe visual impairment. Experimental autoimmune uveitis (EAU) is a preclinical model that shares many features of the human disease and can be induced in rodents by transfer of autoantigen specific lymphocytes. Combined high resolution clinical imaging and flow cytometric assessment, enables us to delineate the ratio of transferred and endogenous cells throughout the disease course. As the sole receptor for CX3CL1, Cx3Cr1 expression is associated with the retention and survival of CD4$^+$ T cells in the lung and skin.
To interrogate the induction of EAU by adoptive transfer of antigen-specific lymphocytes and the role of CX3CR1, cells obtained from immunized CX3CR1$^{+/GFP}$ reporter mice (Ly5.2) were transferred into naïve Ly5.1 recipients. Transferred and endogenous cells rapidly accumulate in the eye within 48 hours. As clinical disease progresses to peak (day 7), 20% of the transferred CD4+ cells were GFP positive, increasing to 50% by day 14. This demonstrates selective enrichment, as the transferred cells comprised an increasing fraction of total retinal cell infiltrate.

These studies suggest that disease-causing antigen specific cells have a long half-life in ocular tissue and/or recirculate extensively. Within the eye, a subset of CD4 cells have a tissue specific phenotype defined by the expression of CX3CR1 which promotes survival or increases retention of specific cells. Long-lived disease-causing cells are therefore a potential candidate for targeted immune-intervention.

P.C1.01.18

Polymorphisms of the cytokine genes TGFB1 and TNFA in Polish patients with inflammatory bowel disease

E. Zakoscielna[1], *M. Zagozda*[1], *A. Surowiecka-Pastewka*[1,2], *M. Durlik*[1,2];
[1]*Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland,* [2]*Central Clinical Hospital of the Ministry of the Interior and Administration, Warsaw, Poland.*

Introduction: Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract, consisting two clinical entities: Crohn's disease and ulcerative colitis. TGF-β and TNF-α are two major immunoregulatory cytokines and their signaling pathways play a critical role in IBD development and progression. The aim of the study was to analyze the TNFA and TGFB1 gene polymorphisms in Polish patients with IBD and its relationship with the secreted cytokines.
Materials and Methods: A total of 42 patients diagnosed with IBD were examined for the TNFA (-308G>A) and TGFB1 (+29C>T) SNP. As a control group, 100 healthy individuals from the same geographical region were selected. Genotyping was performed with the TaqMan assay. Cytokine levels were measured by ELISA in serum samples.
Results: A significantly increased level of TNF-α was observed in patients carrying AA variant of -308G>A polymorphism compared to control. Genotype-phenotype correlation analysis revealed that AA variant was also associated with the development of perianal fistulas. While TGF-β serum level in IBD patients was significantly higher than controls, no significant difference in TGF-β levels between different genotypes exist. Further analysis showed that patients having AA variant of +29C>T SNP suffer from severe disease condition.
Conclusions: Our data suggest that TNFA gene polymorphism participate in determination of IBD susceptibility in Polish patients. The cytokine level is significantly modulated in patients with different genotypes of TNFA gene. Further studies of additional SNPs and increasing the number of individuals should be performed to confirm the role of TNF-α and TGF-β.

P.C1.01.19

Expression of HPGD in regulatory T cells prevents adipose tissue inflammation and metabolic dysfunction

L. M. Schmidleithner[1,2], *Y. Thabet*[2], *E. Schönfeld*[2], *M. Köhne*[1,2], *Z. Abdullah*[3], *K. Klee*[2], *T. Sadlon*[4], *W. Krebs*[2], *K. Subbaramaiah*[5], *M. Schneeweiß*[2], *J. Sander*[2], *N. Ohkura*[6], *A. Waha*[7], *T. Sparwasser*[8], *I. Förster*[2], *H. Weighardt*[2], *S. Sakaguchi*[6], *M. Blüher*[9], *A. J. Dannenberg*[5], *N. Ferreirós*[10], *L. J. Muglia*[11], *C. Wickenhauser*[12], *S. C. Barry*[4], *J. L. Schultze*[2,13], *M. D. Beyer*[1,2];
[1]*German Center for Neurodegenerative Diseases, Bonn, Germany,* [2]*Life and Medical Sciences (LIMES) Institute, Bonn, Germany,* [3]*Institute for Experimental Immunology, Bonn, Germany,* [4]*Robinson Research Institute, Adelaide, Australia,* [5]*Weill Cornell Medical College, New York, United States,* [6]*WPI Immunology Frontier Research Center, Osaka, Japan,* [7]*University Hospital Bonn, Bonn, Germany,* [8]*Institute of Infection Immunology, Hannover, Germany,* [9]*University of Leipzig, Leipzig, Germany,* [10]*Pharmazentrum Frankfurt/ZAFES, Institute of Clinical Pharmacology, Frankfurt, Germany,* [11]*Cincinnati Children's Hospital Medical Center, Cincinnati, United States,* [12]*Institute for Pathology, Martin-Luther University Halle, Halle, Germany,* [13]*German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany.*

Regulatory T cells (T_{reg} cells) are important for the prevention of autoimmunity and lately, their role in maintaining tissue homeostasis has been demonstrated. They exert their function via different suppressive mechanisms including soluble factors. However, how T_{reg} cells exert their function in tissue specific environments is often unknown.
Here, we show that T_{reg}-cell specific expression of hydroxyprostaglandin dehydrogenase (HPGD), which catabolizes prostaglandin E_2 (PGE_2) into 15-keto PGE_2, enforces a new suppressive mode of action through accumulation of the PPAR-γ ligand 15-keto PGE_2. PPAR-γ-dependent HPGD expression acts as the critical molecular link between prostaglandin metabolism, adipose tissue (AT)-associated T_{reg}-cell function, and maintenance of AT homeostasis. In mice, loss of HPGD results in increased numbers of non-functional T_{reg} cells, which accumulate in visceral adipose tissue resulting in increased local inflammation and systemic insulin resistance. In line with this, we could show that in type 2 diabetes (T2D) patients the peripheral blood T_{reg}-cell compartment was reduced and exhibited decreased HPGD expression, indicating that HPGD also seems to be involved in the development of T2D in humans.
These data support that Hpgd is critical for T_{reg}-cell functionality both *in vitro* and *in vivo* and that HPGD acts as a novel tissue- and context-dependent suppressor mechanism by T_{reg} cells to maintain adipose tissue homeostasis.

P.C1.01.20

T-cell dynamics in *Heligmosomoides polygyrus* infection revealed by a new Timer approach

C. B. Ducker[1], *D. Bending*[2], *M. E. Selkirk*[1], *M. Ono*[1];
[1]*Imperial College London, London, United Kingdom,* [2]*University of Birmingham, Birmingham, United Kingdom.*

Heligmosomoides polygyrus (*H. polygyrus*), an intestinal nematode parasite, induces regulatory T-cells (Tregs) to persist within the murine host through secreted effector proteins. However, it is currently unknown whether and how this differs between infection phases, due to a lack of methods to study Treg dynamics and the T-cell response within the periphery. *Timer of cell kinetics and activity* (Tocky) technology allows analysis of the real-time dynamics and history of gene transcription. Here we analyse both *Foxp3*-Tocky and *Nr4a3*-Tocky mice, investigating *Foxp3* expression and TCR signalling respectively in Treg differentiation during different stages of *H. polygyrus* infection.
Nr4a3-Tocky showed that the majority of T-cells were unresponsive during the early phase of infection, while the T-cell response increased when parasites migrate to the intestinal lumen. *Foxp3*-Tocky mice revealed that de novo transcription of *Foxp3* increases in $CD4^+$ T-cells by day 7 of infection. These cells appear to then sustain *Foxp3* transcription, becoming Treg. At day 28 of infection, *Foxp3* transcription is modestly re-instated in a population. We further analysed transcriptomic responses of Tocky populations by RNA-seq.
In conclusion, two individual Treg responses appear to be occurring during *H. polygyrus* infection, depending on the phase. An initial, most likely parasite-mediated response is followed by a response of the major Treg population, possibly as a result of tissue damage. Furthermore, investigation of TCR signalling suggests a potential break in T-cell non-responsiveness during parasite migration.
Funding: BBSRC, MRC

P.C1.02 Maintenance and local regulation of tissue specific immunity - Part 2

P.C1.02.01

Impaired immunomodulatory function of Treg-derived exosomes in RRMS patients

M. Azimi Mohamadabadi, *M. Izad;*
Tehran university of medical science, Tehran, Iran, Islamic Republic of.

Introduction: Multiple sclerosis is an autoimmune disease which is characterized by neuroaxonal degeneration in central nervous system. Impaired function of regulatory T cells (Tregs) is believed to be an underlying pathogenic mechanism in MS. Tregs is able to release exosomes, which contain a considerable amount of protein and RNA. Exosomes are capable of transporting their content to other cells where the released content exerts biological functions. Here, we investigated whether Tregs-exosomes of RRMS patients or healthy controls might regulate the proliferation or survival of T lymphocytes.
Methods: Regulatory T cells derived from MS patients or healthy controls were cultured for 3 days and exosomes were purified from supernatants. Treg-derived exosomes were co-cultured with conventional T cells (Tconv). The percentages of Tconv proliferation and apoptosis were measured.
Result: Our findings showed that the percentage of proliferation suppression induced by exsosomes in patients compared to healthy controls was 8.04±1.17 and 12.5±1.22, respectively; p-value=0.035. Moreover, the rate of Tconv apoptosis induced by exosome of MS patient was less than healthy controls (0.68±0.12 vs. 1.29±0.13; p-value=0.015). Overall, Treg-derived exosomes from MS patients and healthy controls suppressed the proliferation and induced apoptosis in Tconv. However, the effect of MS-derived exosomes was significantly less than healthy controls.
Conclusion: Our results point to an alternative Treg inhibitory mechanism which might be important in immunopathogenesis of MS. Although, the cause of the exosomal defect in MS patients is unclear, manipulation of patients' Treg- derived exosomes to restore their suppressive activity might be considered as a potential therapeutic approach.

P.C1.02.02

The blockage of NMDAr decrease the inflammatory response of T helper cells in an animal model of Multiple Sclerosis

W. N. Brandão[1], *A. C. Durão*[2], *T. T. Braga*[3], *C. Longo*[1], *C. Polinio*[1], *N. Ghabdan*[1], *L. De Oliveira*[1], *T. Marcourakis*[2], *J. S. Peron*[1];
[1]*Institute of Biomedical Sciences, São Paulo, Brazil,* [2]*Faculty of Pharmaceutical Sciences - University of São Paulo, São Paulo, Brazil,* [3]*Federal University of Paraná, Curitiba, Brazil.*

Multiple Sclerosis (MS) consists of an autoimmune disease that have its pathology due to an infiltration of immune cells in the central nervous system (CNS) promoting inflammation and death of resident cells. The factors that influence this disease are not full understanding, especially the effects of neurotransmitters on infiltrated cells. Data from our group shows that glutamate is involved in this pathology and the blockade of its receptor NMDA by MK801 in mice decreases both disease and pathogenic T cells frequency in the CNS. However, is not clear if this phenomenon is dependent of lymphocytes or cells of the innate immune response. In order to overcome this, we developed in our laboratory a model of mice that have the NMDAr blocked only in the T lymphocytes, through the crossing of C57BL/6 $CD4^+$ Cre mice with C57BL/6 Grin1 Flox. Our data shows a delay in the clinical scores of animals knockout for NMDAr in T lymphocytes. This result is associated with lower production of proinflammatory cytokines (IFN-γ and IL-17) by the leukocytes present in the draining lymph nodes during the antigen presentation period (7 days after immunization). In addition, we find a smaller amount of T helper lymphocytes present in these organs, this may be due to a lower proliferative capacity of these cells. Lastly, our data point to a direct effect the downstream of the NMDAr on the T helper lymphocyte response. Financial support CAPES

P.C1.02.03

Influence of Invariant chain and HLA-DM on the HLA-DR3 peptidome constitution

R. Farriol[1], *Y. Arribas*[1], *C. Guitart*[1], *L. Labeur*[1], *V. Casas*[2], *M. Carrascal*[2], *D. Jaraquemada*[1];
[1]*Institute of Biomedicine and Biotechnology, Autonomous University of Barcelona, Cerdanyola del Vallès, Spain,* [2]*CSIC UAB Proteomics Laboratory, CSIC-IIBB, Cerdanyola del Vallès, Spain.*

Genetic-wide-association-studies (GWAS) have pinpointed a high risk to several autoimmune diseases including type 1 diabetes, associated to certain HLA-class-II haplotypes, of which DR alleles HLA-DRB1:04:01 (DR4) and HLA-DRB1:03:01 (DR3) stand out. In a previous study, our group showed the influence of the Invariant Chain (Ii) and HLA-DM on the DR4-peptidome using a rat insulinoma cell line (RINm5f) trnsfected with DR4, Ii and HLA-DM human genes. A similar panel of four transfected cell lines (RIN -DR3DMIi, -DR3DM, -DR3Ii and -DR3) was used to dissect the individual and coordinated effects of these chaperones on the DR3-peptidome. The results showed that both Ii and DM influenced most of the peptidome characteristics, including peptide length distribution, DR3 motif compliance, predicted binding affinity and preference for endocytic degradation. In contrast, the subcellular origin of the proteins represented in the DR3-associated peptidome was independent of chaperone expression. Thus, one third of the peptides from the DR3 repertoire from all the transfectant cells derived from cytosolic degradation, i.e. were either from cytosol or nuclear proteins. The DR3-repertoire from a homozygous human B-cell line showed around 15% of such peptides. These results differ from those from the DR4 transfectants, where the frequency of cytosol-degraded peptides was only high in the HLA-DR4 single transfectants and the expression of Ii and DM normalized the subcellular origin distribution. The data thus suggested an allele dependent feature of the DR3-associated peptidome.

P.C1.02.04

Increased costimulatory molecule expression in thymic and peripheral B cells and a sensitivity to IL-21 in myasthenia gravis

M. HOCAOGLU*, H. DURMUS, B. ÖZKAN, S. YENTUR, O. DOGAN, Y. PARMAN, F. DEYMEER, G. SARUHAN-DIRESKENELI;*
ISTANBUL UNIVERSITY ISTANBUL MEDICAL FACULTY, ISTANBUL, Turkey.

15.00 Normal 0 21 false false false TR X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Normal Tablo"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0cm 5.4pt 0cm 5.4pt; mso-para-margin-top:0cm; mso-para-margin-right:0cm; mso-para-margin-bottom:10.0pt; mso-para-margin-left:0cm; line-height:115%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:"Calibri",sans-serif; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-bidi-font-family:"Times New Roman"; mso-bidi-theme-font:minor-bidi;} Introduction B cells may contribute to the pathogenesis of myasthenia gravis with anti-acetylcholine antibodies (AChR+ MG) and by co-stimulation or selection of T cells. Thymus is a possible site of sensitization for AChR and production of autoreactive antibodies. In this study, we investigated co-stimulatory molecules on B cells as well as the response to innate or adaptive stimulations in AChR+ MG patients with or without immunosuppressive treatment and controls. Methods For this study, 45 AChR+ MG and 21 healthy controls were recruited. Also 27 thymic samples (21 AChR+ MG and 6 Non-MG) were analyzed alongside with blood samples. Peripheral blood and thymic samples were stained with CD19, CD27, CD80, CD86, CD40, HLA-DR antibodies both before and after stimulation with TLR9 agonist and IL-21 and analyzed by flow cytometry. Results CD80 and CD86 expressions on B cells were increased in the peripheral blood and in the thymus of untreated patients. Also thymic B cells had increased CD80 and CD86 compared to peripheral blood samples in both treated and untreated patients. CD86 was further amplified by IL-21 in untreated patients. CD80 expression however did not change. Conclusion A role for activated B cells, active thymic environment and IL-21 is implicated in MG.

P.C1.02.05

Protein YKL-40 and neuron-specific enolase in traumatic brain injury

M. Kazakova[1], *G. Pavlov*[2], *K. Simitchiev*[3], *P. Timonov*[4], *V. Dichev*[5], *C. Stefanov*[2], *V. Sarafian*[5];
[1]*Medical University - Plovdiv, Plovdiv, Bulgaria,* [2]*Medical University - Plovdiv; 2Department of Anesthesiology, Emergency and Intensive Care Medicine; University Hospital "St. George", Plovdiv, Bulgaria,* [3]*3Department of Analytical Chemistry and Computer Chemistry, Faculty of Chemistry, University of Plovdiv, Plovdiv, Bulgaria,* [4]*Department of Forensic Medicine, Medical Faculty,Medical University – Plovdiv, Plovdiv, Bulgaria,* [5]*Department of Medical Biology, Medical Faculty, Medical University – Plovdiv, Technological Center for Emergency Medicine, Plovdiv, Bulgaria.*

Introduction: Traumatic brain injuries (TBIs) are major issue of public health. The glycoprotein YKL-40 is expressed as a consequence of a broad spectrum of inflammatory and malignant diseases. Neuron-specific enolase (NSE) is considered as a biomarker of neuronal stress and inflammatory conditions.
The purpose of this investigation is to determine the dynamics of NSE and YKL-40 levels after TBI.
Methods: We examined plasma and cerebrospinal fluid (CSF) levels of both proteins in nineteen patients – on the 24th and 96th hour after the TBI. Routine haematological and biochemical tests were also performed. As control served the CSF of age-matched suddenly deceased healthy individuals.
Results: We found no significant change between CSF levels of YKL-40 and NSE on the 24th h after TBI compared to the control group. However the interquartile range (25–75 percentile) of the YKL-40 concentrations for the control group was much lower than the one of TBI patients. NSE levels in CSF on the 24th h were higher in comparison with plasma concentrations (p=0.001), while no difference on the 96th h was observed.
Significant difference between plasma and CSF YKL-40 levels on the 24th (p=0.027) and 96th (p=0.044) in the patients with TBI was detected. No association of YKL-40 and NSE concentrations with monocytes and neutrophils counts (as percentages of WBC) was observed.
Conclusion: We assume that YKL-40 levels reflect better the inflammatory process in TBI. Acknowledgements: The financial support by the National Science Fund of Bulgaria (Contract DM 03/2 12.12.2016) is gratefully acknowledged

P.C1.02.07

Diet-induced obesity alleviates collagen-induced arthritis (CIA) in male DBA/1 male mice

K. Marcińska, ***P. Kowalczyk****, A. Strzępa, M. Majewska-Szczepanik, M. Szczepanik;*
Department of Medical Biology, Faculty of Health Sciences, Jagiellonian Univeristy Medical College, Krakow, Poland.

At present there are only a few reports concerning the effects of obesity on collagen-induced arthritis (CIA) in mice and rheumatoid arthritis in patients. Studies conducted by our team demonstrated that diet-indecent obesity (DIO) aggravates T-cell mediated immune response such as contact hypersensitivity in mice. Unexpectedly, our preliminary data employing CIA model showed that male mice with DIO develop less severe disease. To study the influence of obesity on CIA, DBA/1 mice were fed high fat diet (HFD) or normal diet for 4 or 8 weeks prior to CIA induction. Our data show that male but not female mice fed HFD for 4 or 8 weeks develop less severe disease (clinical score). Decreased disease severity in male mice correlated with reduced myeloperoxidase (MPO) activity in joint tissue. Moreover, reduced concentration of anti-COLL II IgG2a correlated with decreased disease severity in HFD-fed male mice. Observed reduction of anti-COLL II IgG2a in male HFD-fed mice is in line with decreased percentage of $CD19^+$ $B220^+$ B cells in the spleen when compared to female mice fed HFD. Further flow cytometry analysis of lymphoid organs showed increased percentage of $CD4^+IL10^+$ lymphocytes in the spleen of HFD-fed male mice that developed less severe disease. This was not observed in female mice with DIO. In summary, our data show that DIO alleviates CIA in male but not female mice suggesting that this phenomenon is determined by gender. This work was supported by grant K/ZDS/007122 to MS.

P.C1.02.08

Genetic variants in HLA and C11orf30 locus associated with breach of Immune tolerance and autoantibody production in healthyand SLE

Q. Li*, P. Raj, H. Zhu, I. Dazmorov, D. Karp, E. K. Wakeland;*
University of Texas Southwestern Medical Center, Dallas, United States.

Molecular dissection of the genetic variants from 3,903 healthy and autoimmune individuals with different levels of autoantibodies (autoAbs) revealed two major genetic loci in HLA and C11orf30 regions which are highly associated with break in immune-tolerance and autoAb production. The variants in HLA locus associated with IgG autoAbs was the same SLE risk haplotype that impact consensus binding motifs of IRF4 and CTCF in the XL9 regulatory complex and modify the transcription of MHC class-II antigen presenting genes. The HLA haplotype 3 (HAP3), which was identified to be a SLE risk haplotype, was associated with ANA positivity in healthy individuals (OR=2.1, p=0.01). High resolution autoantibody profiling showed an increased level of autoAbs against non-nuclear antigens in ANA positive healthy and incomplete lupus, while high titer of autoAbs targeting to nuclear antigens were present in most lupus patients, suggesting a target transition of IgG autoAbs during the disease development. Variants in C11orf30 locus associated with ANA in healthy but not in SLE. The carriers of C11orf30 risk haplotype demonstrated strong titers of IgG and IgA autoAbs to many common allergens i.e. cedar Red juniper pollen and whole wheat triticum. The risk haplotype of C11orf30 was associated with downregulation of its expression in EBV cells and MDMs. Whole transcriptomic analysis of B cells from ANA positive healthy donors carring the risk alleles revealed significantly upregulated BTK, SLAMF1&6, BLNK, FCRL1 and HLA-DRB1 genes, suggesting enhanced B cell signaling.

P.C1.02.09

Circulating IL-10 producing B cells in CSF of neuroimmunological disorders patients

O. Maghrebi[1], *M. belghith*[2], *K. Bahrini*[3], *S. ben Sassi*[4], *S. Blel*[4], *M. R. Barbouche*[3];
[1]*Institut Pasteur de Tunis, Belvédère Tunis, Tunisia,* [2]*Institut Pasteur de Tunis, tunis, Tunisia,* [3]*Institut Pasteur de Tunis, Belvédère tunis, Tunisia,* [4]*Institut Mongi Ben Hmida de Neurologie De Tunis, Tunis, Tunisia.*

Introduction/objective: Neuroimmunological disorders are a spectrum of diseases characterized by a chronic neuroinflammation causing neurologic lesions. The recurrent clinical course of the majority of those diseases suppose an interplay between self-reactive and immunoregulatory process. T and B cells are generally considered as effectors cells, but it is now clear that they are essential for inducing immune tolerance by regulating immune responses via IL-10 production. The aim of this work is to assess involvement of different cell subsets secreting Il-10 in the cerebrospinal fluid of patients with neuroinflammatory disease. **Material /Methods:** Blood and cerebrospinal fluid (CSF) samples from patients with CNS inflammatory diseases were collected at time of clinical relapse. Ethical clearance and written consent were obtained for all of them. Isolated PBMCs and CSF cells were immunostained with a combination of specific antibodies and then analyzed using cytometry. **Results/ conclusion:** Our results show IL-10 higher expression in CSF neuro-inflammatory diseases like Neuro-Behçet compared to neuro-autoimmune disorders. The evaluation of the eventual source of this cytokine show us an increased IL-10 producing B cells in CSF of patients compared to IL-10 producing CD4. Interestingly, the CD4 subset secretes lower levels of IL-10 in the two studied compartments of patients compared to healthy controls. In blood there was no significant difference between the two studied cells in their capacity to produce IL-10. In summary, our current findings confirm the deleterious IL-10 production by TCD4 subsets and suggest that IL-10 producing B cells are the major regulatory cells involved in neuroinflammatory environment.

P.C1.02.10

Role of the complement system in Sjögren syndrome

M. Masarwa[1], *I. Alon*[2], *A. Pikovsky*[3], *A. Livoff*[2], *B. Forer*[1];
[1]*The Department of Otolaryngology-Head and Neck Surgery, Barzilai University Medical Center (Affiliated to the Faculty of Medicine, Ben Gurion University of the Negev, Beer Sheva), Ashkelon, Israel,* [2]*The Department of Pathology, Barzilai University Medical Center, Ashkelon, Israel,* [3]*The Unit for Oral Medicine, Barzilai University Medical Center, Ashkelon, Israel.*

Sjögren syndrome (SS) is a progressive autoimmune disease characterized by a wide range of symptoms. Most patients present with mild sicca symptoms but others may develop serious systemic manifestations including vasculitis and severe vital organ dysfunction. The main pathophysiologic basis of primary SS is attributed to tissue-infiltrating T and B cell lymphocytes, triggered by concomitant inflammatory mediators and chemotactic agents. Though low levels of circulatory complement proteins are frequently observed in SS, especially in the extra-epithelial form of the disease, little is known about the contribution of the complement system to the pathogenesis of the disease. The aim of this study is to investigate the role of complement in the pathogenesis of SS.
Deposition of complement C3 and C9 proteins was examined in minor salivary gland specimens excised from SS patients (n = 16). Oral biopsies taken because of other non-inflammatory lesions and contained incidental minor salivary glands, served as controls (n = 6). Complement C3 and/or C9 deposition was observed in 93% of SS patient specimens, in contrast to 17% of controls. The sensitivity and specificity of the test herein was 93% and 83% respectively. Complement deposition was mostly seen around the ducts or within the ductal cells.
To our knowledge, this is the first time complement deposition was found in salivary glands of SS patients. These findings may pave the way for the development of new anti-complement therapeutic agents for patients with SS.

P.C1.02.11

Jmjd6 controls Aire protein expression and self-tolerance induction in the thymus

K. Matsubara[1], *T. Yanagihara*[1], *Y. Fukui*[1,2];
[1]*Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan,* [2]*Research center for Advanced Immunology, Kyushu university, Fukuoka, Japan.*

Introduction: To establish immunological self-tolerance in the thymus, medullary thymic epithelial cells (mTECs) express diverse sets of tissue-specific self-antigens (TSAs). This ectopic expression of TSAs largely depends on the transcriptional regulator Aire, yet the mechanism controlling Aire expression itself remains unknown. In this study, we examined the role of Jmjd6, a lysyl hydroxylase for splicing regulatory proteins, in Aire expression.
Material and methods: As conventional Jmjd6 KO mice die during perinatal period, 2DG-treated fetal thymi were prepared from the KO mice and control mice for FACS analyses, immunohistochemical analyses and RNAseq analyses. The 2DG-treated fetal thymi from these mice were grafted into C57BL/6 nude mice to examine its effect on self-tolerance induction. The conditional Jmjd6 KO mice (Foxn1-CRe Jmjd6$^{lox/lox}$ mice) were crossed with a diabetes mouse model (OT1+RIP-mOVA+ mice) to analyse specific tolerance induction in a more physiological condition.
Results: Although Jmjd6 deficiency did not affect abundance of *Aire* transcript in mTECs, the intron 2 of *Aire* gene was not effectively spliced out in the absence of Jmjd6. As a result, the expression of mature Aire protein and TSAs were markedly reduced in Jmjd6-deficient mTECs, and C57BL/6 nude mice grafted with Jmjd6-deficient thymi spontaneously developed multi-organ autoimmunity. In addition, we found that TEC-specific deletion of Jmjd6 exacerbated development autoimmune diabetes in a mouse model.
Conclusion: Jmjd6 expression is required in mTECs for expression of mature Aire protein and induction of self-tolerance in developing T cells.

P.C1.02.12

Role of the transcription factor Eomes in CD4 T cell functions and susceptibility to CNS autoimmunity

M. F. Michieletto[1,2,3], *E. Joulia*[1,3,2], *V. Girault*[1,3,2], *A. Saoudi*[1,3,2], *A. S. Dejean*[1,3,2];
[1]*INSERM U1043, TOULOUSE, France,* [2]*Paul Sabatier University, Toulouse, France,* [3]*CNRS UMR 5282, Toulouse, France.*

T cell responses play a crucial role in inflammation of the central nervous system (CNS) such as Multiple Sclerosis (MS). However, the molecular mechanisms conferring encephalogenic properties to CD4 T cells remain unclear. Using the Experimental Autoimmune Encephalomyelitis model (EAE), we showed that the Foxo3/Eomes axis controls pathogenic CD4 T cell polarization and susceptibility to autoimmunity. Indeed, Foxo3KO mice were resistant to EAE due to the inability of CD4 T cells to differentiate into IFN-g and GM-CSF secreting cells. At the molecular level, we identified Eomes as a direct target gene of Foxo3. *EOMES* is a susceptibility gene in MS, however, the specific functions of this transcription factor in CD4 T cells require in-depth characterization. EomesKO renders mice resistant to EAE and T cell transfer experiment showed that EomesKO CD4 T cells exhibit decreased encephalogenic properties. Using Eomes-GFP reporter mice, we showed that Eomes$^+$ CD4 T cells accumulates over time during EAE, produced more proinflammatory cytokines and displayed specific migratory capacity. *In vitro*, we showed that Eomes is expressed by a specific population of long-lived effector CD4 T cells with peculiar functions. In Human, we showed that EOMES is mostly expressed by Effector and Effector Memory CD4 T cells. Expression of EOMES allowed CD4 T cells to respond faster upon activation and to secrete high amount of pro-inflammatory cytokines such as IFN-g, GM-CSF and decreased amount IL-4 and IL-17. Altogether, our results suggest that Eomes expression might define a specific subpopulation of Th cells playing a key role in neuroinflammation.

P.C1.02.13

Immunopathogenesis of vitiligo: do cytotoxic CD8$^+$ T-cells have a role?

S. Mitra, *S. Mukherjee, S. Sengupta, A. K. Pati, A. Ghosh, S. Sen, U. Chatterjee, S. Chatterjee, M. Chatterjee;*
Institute of Post Graduate Medical Education and Research, KOLKATA, India.

Introduction: Vitiligo, an autoimmune disease is characterized by melanocyte destruction and affects 0.5-1.0% of the world population. An altered immunological milieu may contribute to disease pathogenesis and this study aimed to evaluate the status of CD8$^+$ T-cells in patients with vitiligo.
Materials and Methods: The status of CD8$^+$ T-cells in peripheral blood was evaluated in terms of Granzyme B positivity and propensity to dermal homing (CXCR3) by flow cytometry. Concomitantly, levels of circulating pro-inflammatory cytokines (IL-2, IL-6, TNF-α, IFN-γ, IL-1ß, IL-8) and chemoattractants (CXCL9, CXCL10, CXCL11) were measured using a multiplex assay. The presence of dermal CD8$^+$ T-cells was evaluated by immunohistochemistry.
Results: In peripheral blood of patients with vitiligo as compared to healthy individuals, CD8$^+$ T-cells demonstrated an increased frequency of Granzyme B and CXCR3. Additionally, levels of circulatory pro-inflammatory cytokines (IL-6, TNF-α, IL-1β, IFN-γ and IL-8), and chemokines (CXCL9, CXCL10) was enhanced. An increased presence of epidermal CD8$^+$ T-cells was demonstrated in the perilesional skin of patients with vitiligo.
Conclusions: Patients with vitiligo had higher levels of pro-inflammatory cytokines (IL-6, TNF-α, IL-1β, IFN-γ and IL-8). The increased levels of IFN-γ possibly caused upregulation of CXCR3 in CD8$^+$ T-cells, and the concomitant increment in the levels of chemokines, CXCL9 and CXCL10 facilitated the migration of CD8$^+$ T-cells to the dermal milieu which may be responsible for the destruction of melanocytes, a hallmark of the disease.
Funding:
•Dept. of Science & Technology (DST), Govt. of India & Govt. of West Bengal
•Fellowship INSPIRE Programme DST, Govt. of India.

P.C1.02.14

Descriptive analysis of patients with antibodies related to systemic sclerosis

M. San Jose-Cascon[1], *A. Pérez Linaza*[2], *R. de la Varga Martínez*[3], *E. Velazquez*[4], *F. Medina Varo*[2], *C. Rodríguez*[2];
[1]*UGC Hematology, Immunology and Genetics, Hospital Universitario Puerta del Mar, Cadiz, Spain,* [2]*Hospital Universitario Puerta del Mar, Cadiz, Spain,* [3]*Hospital Universitario Virgen del Rocío, Sevilla, Spain,* [4]*Hospital Dr. Arturo Oñativia, Buenos Aires, Argentina.*

Abstract: Systemic sclerosis (SSc) is an autoimmune disease characterized by cutaneous and visceral fibrosis associated to small-vessel vasculopathy. There is evidence that both capillary anomalies and the course of disease are associated to the presence of specific autoantibodies (Ab) such as anti-centromere Ab (CENP-Ab) and anti-topoisomerase I Ab (Scl-70).
Objective: Our aim was to evaluate the diagnostic and prognostic value of CENP and Scl-70 Ab in patients diagnosed with SSc from our hospital.
Patients and methods: Patients with specific Ab of SSc were retrospectively selected from January-2012 to December-2017 (n=70). Clinical and laboratory data were collected and patients were classified according to ACR/EULAR 2013 criteria. The association between Ab and clinical parameters were assessed in the group of patients diagnosed with SSc (n=27).
Results: Among 70 patients, 58 had CENP-Ab and 12 had Scl-70 Ab. 2/3 of patients with SSc presented the limited form and the most frequent clinical manifestations were Raynaud phenomenon, digestive complaints and telangiectasias. Digital ulcers were observed in 9% of CENP-Ab vs 100% of Scl-70 positive patients ($p<0.05$, $\chi 2$). Lung involvement was present in 54% of patients with CENP-Ab vs 100% of patients with Scl-70 ($p<0.05$, $\chi 2$). Patients with anti-Scl-70 had higher levels of ESR than those with CENP Ab ($p<0.05$, $\chi 2$).
Conclusions: The presence of Scl-70 was associated with more frequent lung involvement, digital ulcers and higher levels of ESR, whereas CENP-Ab appeared in limited SSc. Therefore, anti-Scl-70 Ab are laboratory markers for identifying SSc patients with worse prognosis.

P.C1.02.15

Age related macular degeneration and innate immunity: molecular crosstalk between the complement system and the long pentraxin 3

M. Stravalaci[1,2], *M. R. Romano*[1], *F. Petroni*[2], *R. Parente*[2], *M. Gobbi*[3], *B. Bottazzi*[2], *A. Mantovani*[1,2], *A. Inforzato*[1,2];
[1]*Department of Biomedical Sciences, Humanitas University, Pieve Emanuele (Milano), Italy,* [2]*IRCCS-Humanitas Clinical Research Institute, Rozzano, Italy,* [3]*IRCCS-"Mario Negri" Institute for Pharmacological Research, Milano, Italy.*

Dysregulation of the complement system is a key event in age-related macular degeneration (AMD), the leading cause of blindness in the developed world. Most of the genetic variation associated with AMD resides in genes encoding complement proteins, with the greatest risk being contributed by factor H (FH), major inhibitor of the alternative pathway of complement (AP). FH regulates complement activation both in solution and on cell surface by mediating the proteolytic cleavage of C3b, activator of the AP. One of the ligands of FH is the long pentraxin 3 (PTX3), a soluble pattern recognition molecule involved in innate immunity, tissue homeostasis and inflammation. PTX3 is locally produced in the retina pigmented epithelium (RPE), where, based on mouse models of AMD, it is believed to modulate complement activation via recruitment of FH. However how this occurs in the human eye is yet to be defined. In the present work we employed an integrated approach using *in vitro* binding assays and a cell model of RPE (ARPE-19) to investigate the PTX3-FH-C3b interactions and their effect on AP activation. In solid phase and surface plasmon resonance binding experiments we found that PTX3 specifically recognizes C3b, and this interaction is strengthened by FH. Importantly, C3b could not trigger AP activation when bound to PTX3. We extended these investigations to ARPE-19 cells under either oxidative or inflammatory conditions, to model the human disease *in vitro*. These findings point to a novel mechanism of complement regulation by PTX3 with potential implications in the etiopathogenesis of AMD.

P.C1.02.16

Human kidneys house tissue-resident B cells with a distinct phenotype and anatomical location

O. Suchanek, *J. Ferdinand, K. Loudon, A. Riding, M. R. Clatworthy;*
MRC Laboratory of Molecular Biology, Molecular Immunity Unit, University of Cambridge, United Kingdom.

Background: B cells play a central role in humoral immunity via antibody production but have also antibody-independent functions. There is an increasing appreciation of the importance of tissue-resident immune cells in generating local immune responses. To date, the question of whether B cells reside in non-lymphoid organs has received little attention. **Methods:** We examined the number, phenotype and clonality of B cells in human kidneys that were perfused to remove circulating cells, and in splenic tissue obtained from the same donor (N=10, median age 56 years (range: 23-80)). Percolled single cell suspensions from homogenized organs were analyzed using a 35-marker mass cytometry panel, and B cells were also sorted for RNA-sequencing. **Results:** The composition and size of the major renal leukocyte populations was heterogeneous between donors. The frequency of B cells in both the renal cortex and medulla was lower than that observed in spleen (8.8% and 5.8% vs. 26%, P<0.005). The renal cortex harbored five times more B cells per gram of tissue than medulla (P 0.02). Both renal cortex and medulla were significantly enriched for non-naïve B cells compared with spleen. BCR analysis showed CDR3 repertoire differences between kidney and spleen suggesting a specific antigenic exposure. **Conclusion:** Our study shows that under homeostatic conditions, the extravascular compartment of human kidneys harbors antigen-experienced B cells, mirroring studies of murine tissue-resident T cells. These kidney B cells may play a role in local immune defense or contribute to immunopathology and further studies on diseased tissues and murine models are underway.

P.C1.02.17

Highly inflammatory multiple sclerosis patients show an increase in B cell cytokine production in cerebrospinal fluid

A. Tejeda Velarde[1], *E. Rodríguez Martín*[1], *L. Costa-Frossard*[1], *Y. Aladro*[2], *S. Sainz de la Maza*[1], *M. Espiño*[1], *S. Medina*[1], *N. Villarrubia*[1], *E. Monreal*[1], *J. Álvarez-Cermeño*[1], *E. Roldán*[1], *L. Villar*[1];
[1]*Ramón y Cajal University Hospital, Madrid, Spain,* [2]*Getafe University Hospital, Madrid, Spain.*

Introduction: B cells play an important role in multiple sclerosis (MS) physiopathology. This effect may be partly mediated by mechanisms unrelated to immunoglobulin secretion. Lipid-specific IgM oligoclonal bands (LS-OCMB) identify patients with a highly inflammatory MS course. We aimed to study if these patients show different B cell profiles in cerebrospinal fluid (CSF) and peripheral blood, with a special focus in intracellular cytokine production.
Patients and methods: We analyzed CSF and blood samples of 45 relapsing-remitting MS patients. We studied LS-OCMB by isoelectric-focusing and western blot and analyzed B cell subsets and B cell cytokine production in CSF and blood by flow cytometry in a FACSCanto II. Results were expressed as percentage of CD45 mononuclear cells. Differences between patients showing and lacking LS-OCMB were studied using Mann-Whitney U test.
Results: 18% of patients showed LS-OCMB. These patients showed in peripheral blood a decrease in regulatory B cells ($p=0.027$) and a trend to increase in memory B cells ($p=0.051$). In CSF, the presence of these antibodies associated with increases in B cells producing GM-CSF ($p=0.018$), and TNFα ($p=0.048$), being the augment even higher when considering B cells producing both GM-CSF and TNFα ($p=0.007$).
Conclusions: These data show that MS patients with a highly inflammatory disease experience an increase in B cells producing pro-inflammatory cytokines within the central nervous system, thus reinforcing the role of these cells in MS physiopathology.

P.C1.02.18

The relevance of the C5a/C5aR1 axis in production of auto-antibodies in the murine immunization model of Epidermolysis bullosa acquisita

J. Tillmann[1], *B. Kovács*[1,2], *J. Figge*[1], *R. Ludwig*[2], *J. Köhl*[1,3], *C. Karsten*[1];
[1]*Institute for Systemic Inflammation Research, Lübeck, Germany,* [2]*Department of Dermatology, University of Lübeck, Lübeck, Germany,* [3]*Division of Immunobiology, Cincinnati Children's Hospital Medical Center and University of Cincinnati College of Medicine, Cincinnati, Ohio, United States.*

Epidermolysis bullosa acquisita (EBA) is a skin blistering disease caused by autoantibodies (auto-Abs) against collagen typ VII (Col7), one of the major components of anchoring fibrils in the dermal-epidermal junction. Previously, we found that deficiency of C5aR1 protects from disease development in an antibody-transfer model of EBA, demonstrating an important role for C5aR1 in the effector phase of the disease. Here, we show the importance of C5aR1 in an immunization model of EBA and redefine the view on the contribution of Fcγ-receptors (FcγR) to disease development. The active EBA mouse model is induced by subcutaneous immunization with Col7. Mice were scored and induction of Col7-specific auto-Abs was analyzed in wild type (WT) and C5aR1-deficient ($C5aR1^{-/-}$) mice, biweekly. Additionally we determined IgG pathogenicity using ROS-release-assay and assessed IgG Fc-glycosylation, which defines pro- or anti-inflammatory properties of IgGs. We observed first symptoms after 4 to 6 weeks in WT mice, whereas $C5aR1^{-/-}$ mice were protected from EBA. $C5aR1^{-/-}$ showed significantly decreased levels of pro-inflammatory agalactosylated auto-Abs compared to WT. Further, analyzing IgG pathogenicity using ROS-release-assay we found that in contrast to previous reports that FcγRIII and FcγRIV are equally important for activating neutrophils. Our findings identify the C5a/C5aR1 axis as a critical driver of EBA not only in the effector but also in the initiation phase shaping a pathogenic Ab response. Furthermore, our findings show that the previously assumed exclusive dependency on FcγRIV is not valid anymore and needs to be re-evaluated.

P.C1.02.19

Autoimmunity to beta subunit of P4Hb and glucokinase linked to insulin secretion in type 1 diabetes

M. Yang[1], *S. Horstman*[2], *R. A. Ettinger*[2], *C. Speake*[2], *E. A. James*[2], *K. Herold*[1], *M. J. Mamula*[1];
[1]*Yale University School of Medicine, New Haven, United States,* [2]*Benaroya Research Institute, Seattle, United States.*

Introduction: Inflammation and oxidative stress in pancreas amplifies various post-translational modifications (PTMs) on self-proteins. The loss of immune tolerance to PTMs within the stressed islets subsequently impacts the autoreactive T cell epitope repertoire and contributes to the destruction of insulin-producing beta cells in T1D. However, the mechanism of carbonylation and citrullination, common PTMs found in stressed islets, modulates immunogenicity and islets functions is still unclear.
Materials and Methods: Mass spectrometry was performed to map the PTM sites. Humoral responses and immunogenicity to modified islet proteins were evaluated by ELISA and HLA tetramers, respectively. Finally, proinsulin and insulin secretion upon glucose stimulation was examined in human islets in the context of beta cell PTMs.
Results: We identified six carbonyl residues within P4Hb and nineteen citrullination modifications within glucokinase. Carbonylated-P4Hb is amplified in stressed islets coincident with decreased glucose-stimulated insulin secretion and altered proinsulin to insulin ratios. Autoantibodies against both P4Hb and glucokinase arise in human T1D patients. Likewise, CD4+ T cells specific for citrullinated glucokinase are present in the circulation of T1D patients. Mature insulin is stored in the form of Zn-containing hexamers until secretion. Interestingly, anti-glucokinase antibodies correlated with anti-ZnT8, but not with other T1D autoantibodies (Insulin, GAD, IA2).
Conclusions: In beta cells, glucokinase acts as a glucose sensor to initiate glycolysis and insulin signaling. P4Hb is critical for proinsulin/insulin folding. Our studies implicate these crucial enzymes as biomarkers, providing new insights into creating autoantigens and define the impact of PTMs on the biological function of beta cells in T1D.

P.C1.02.20

Human intestinal mucosa contains a large population of CD8 resident memory T cells

R. Bartolome Casado[1], *O. Landsverk*[1], *S. Chauhan*[1], *L. Richter*[1,2], *Y. Yao*[3,4], *V. Greiff*[3], *L. F. Risnes*[3,4], *R. S. Neumann*[3,4], *S. Yaqub*[5], *O. Øyen*[6], *R. Horneland*[6], *E. M. Aandahl*[6], *V. Paulsen*[7], *L. M. Sollid*[3,4], *S. Qiao*[3,4], *E. S. Bækkevold*[1], *F. L. Jahnsen*[1];
[1]*Department of Pathology and Centre for Immune Regulation (CIR), Oslo University Hospital –Rikshospitalet, Oslo, Norway,* [2]*Core Facility Flow Cytometry, Biomedical Center, Ludwig-Maximilians-University Munich, Planegg-Martinsried, Germany,* [3]*Department of Immunology and Centre for Immune Regulation (CIR), Oslo University Hospital –Rikshospitalet, Oslo, Norway,* [4]*K.G. Jebsen Coeliac Disease Research Centre, Oslo University Hospital – Rikshospitalet, Oslo, Norway,* [5]*Department of Gastrointestinal Surgery, Oslo University Hospital - Rikshospitalet, Oslo, Norway,* [6]*Department of Transplantation Medicine, Section for Transplant Surgery, Oslo University Hospital, Rikshospitalet, Oslo, Norway,* [7]*Department of Gastroenterology, Oslo University Hospital - Rikshospitalet, Oslo, Norway.*

Recent studies in mice have described resident memory CD8 T-cells (Trm) as key immune mediators of long-lasting protective responses in non-lymphoid tissues. The gut is a large immune organ and one of the main sites of pathogen entry. However, our knowledge about CD8 Trm in the human gut is very limited. Here we examined the replacement of CD8 T-cell subsets in transplanted small intestine (SI) by flow-cytometric analysis of HLA-I mismatched donors. We found that 60% (n=15) of CD103+ CD8 T-cells both in the epithelium and lamina propria (LP) were of donor origin one year after transplantation, whereas LP CD103- CD8 T-cells were rapidly exchanged (15%, n=15). Single-cell TCR sequencing of donor CD103+ CD8 T-cells from transplanted SI revealed that a significant fraction of expanded TCR clonotypes at baseline were maintained one year after transplantation. The persistent CD103+ CD8 T-cells were CD69+ KLRG1- $CD127^{hi}$ $CD28^{lo}$ $PD1^{lo}$, and this distinctive phenotype was progressively acquired by the incoming recipient CD8 T-cells. LP CD103+ CD8 T-cell clones were highly overlapping with their counterparts in epithelium. Moreover, we found substantial clonal overlap between CD103+ and CD103-KLRG1- CD8 subsets in LP, suggesting that CD103+ CD8+ Trm might derive from CD103-KLRG1- precursors. CD103+ CD8 T cells expressed lower levels of preformed cytotoxic granules but produced more cytokines than the CD103- counterpart. In summary, we provide definitive evidence that most human intestinal CD103+ CD8 T cells persist over long periods of time and show immunogenomic, phenotypic and functional characteristics of murine CD8 Trm.

P.C1.03 Maintenance and local regulation of tissue specific immunity - Part 3

P.C1.03.01

CD39/CD73 implication in neuroimmunological inflammatory disorders

k. BAHRINI[1], *M. Belghith*[1], *O. Maghrebi*[1], *S. Ben sassi*[2], *S. Belal*[2], *M. Barbouche*[1];
[1]*Insitut pasteur de Tunis, Tunis, Tunisia,* [2]*Institut Mongi ben Hamida de Neurologie De Tunis, Tunis, Tunisia.*

Introduction/Objective: Treg cells can be divided in two subsets based on the expression of CD39 an ectonucleotidase that catalyzes the conversion of pro-inflammatory extracellular ATP to adenosine which present a regulatory effect. CD39+ Tregs but not CD39-T regs have the potential to suppress the pathogenic IL-17. The purpose of our study is to characterize the CD39 population in the blood and cerebrospinal fluid (CSF) of Multiple sclerosis and Neuro-behçet disease to determine the role of this ectoenzyme in these two disorders.
Materiel and methods: We quantified, using quantitative RT-PCR, the mRNA expression of IL-10, IL-4, GATA3, Foxp3, CD39 and CD73 in the PBMC and CSF of 21 patients with relapsing remitting multiple sclerosis (RRMS), 19 patients with Neuro-Behçet disease (NBD) and 22 healthy controls. CD39 and CD73 in blood and CSF were studied simultaneously with regulatory markers by flow cytometry.
Results: Our results show no significant difference in the expression of IL-4, GATA3 and Foxp3 mRNA in the blood and CSF of the three studied groups. Concerning CD39 expression, we revealed a significant decrease of CD39 regulatory cells expressing Foxp3 and IL10 in PBMC of RRMS compared to NBD (p<0, 05). Surprisingly, in the CSF we detected a high level of CD39 in RRMS and NBD patients (p<0,001) compared to controls. CSF NBD CD39 cells aren't associated with regulatory markers. However in half of RRMS patients, CD39 is associated with IL10 and CD73. These results demonstrate a differential expression of CD39 depending on the inflammatory CNS Statue.

P.C1.03.02

IL-17 producing iNKT cells correlate positively with disease activity DAS28-ESR in rheumatoid arthritis

K. CHOWDHURY[1], *D. K. Mitra*[1], *U. Kumar*[1], *S. Das*[2];
[1]*All India Institute of Medical Sciences, Delhi, India,* [2]*Albert Einstein College of Medicine, USA., Newyork, United States.*

Rheumatoid arthritis (RA) is well established autoimmune disease mediated through various pathogenic T cells. iNKT cells constitute 0.5% of total NKT cells. The role of iNKT cells in RA animal model (collagen induced arthritis; CIA) is shown to be protective by IL-10 production. On contrary they are also shown to be secretor of IL-17 which is pathogenic to RA. There is no direct evidence of human study which shows the precise role of iNKT in disease pathogenesis or amelioration. So we intend to study CD1d restricted iNKT cells in PBL and SF of rheumatoid arthritis patients. We have recruited treatment naive activate RA patients with DAS28-score>3 (N=23) . Cells derived from peripheral blood and synovial fluid was stimulated with alpha-galactosylceramide (alpha-galcer) for 48 hours to measure cytokine production and proliferation. we have observed higher frequency of IL-17+iNKT+ cells as well as CD1d restricted iNKT in RA SF compared to autologous PBL (*p=0.001*) and positively correlates with DAS28-ESR (R=0.79). CD3+CD161+ NKT cells were also higher in RA SF. Moreover alpha-galcer stimulation up regulated IL-17+ iNKT cells instead of IL-10 in RA SF derived cells. RA synovial CD1d restricted iNKT cells are polarized towards IL-17 production instead of IL-10 upon alpha-galcer stimulation. IL-17+iNKT cells proliferates more in RA SF upon alpha-galcer stimulation and also correlates with DAS28 ESR. Taken together these findings hint towards pathogenic role of iNKT cells in RA.

P.C1.03.03

TIGIT expression identifies gut-homing effector T cells with immunomodulatory properties that are deregulated in inflammatory bowel disease patients

M. E. Joosse, *L. M. Costes, Y. Simons-Oosterhuis, R. C. Raatgeep, L. A. van Berkel, T. Cupedo, L. de Ridder, J. C. Escher, S. Veenbergen, J. N. Samsom;*
Erasmus MC, Rotterdam, Netherlands.

Inflammatory bowel disease (IBD) is characterized by intestinal infiltration of pathologic effector T-cells. The defects driving loss of normal T-cell regulation in IBD remain undefined. We could show that in healthy murine and human intestine 40-50% of gut-resident $CD38^+CD4^+$ effector T-cells express T-cell immunoglobulin and ITIM domain (TIGIT), an inhibitory receptor capable of modulating dendritic cell (DC) and T-cell function. In peripheral blood of healthy individuals, TIGIT expression was enriched on gut-homing $CD38^+$ effector T-cells while conversely, in IBD patients with active intestinal inflammation, frequencies of $CD38^+TIGIT^+$ effector T-cells were decreased and associated with severe disease course. This raised the question whether $CD38^+TIGIT^+$ effector T-cells mediate intestinal immune regulation. Bacterial colonization of germ-free mice and associated induction of regulatory immunity, increased TIGIT expression in intestinal tissue. To assess whether TIGIT is preferentially expressed by proliferating cells in mucosa-draining lymph nodes (LNs), we compared TIGIT expression on ovalbumin (OVA)-specific $CD4^+$ T-cells in murine draining LNs after OVA feed or intramuscular (i.m.) OVA injection. After OVA feed, TIGIT was most prominently expressed on T-cells in celiac LN draining the duodenum and liver when compared to T cells in peripheral LN after OVA i.m. injection. In humans, a high percentage of circulating $TIGIT^+CD38^+$ effector T-cells co-expressed the immune-regulatory molecules PD-1 or CTLA-4. Importantly, $CD38^+TIGIT^+$ effector T-cells modulated DC activation, and co-cultures with immature monocyte-derived DCs induced lower levels of *IL12p35*, *IL23p19* and *IL6* compared to co-cultures with $CD38^+TIGIT^{neg}$ cells. In sum, we have identified $CD38^+TIGIT^+$ effector T-cells as potential regulators of intestinal immunity.

P.C1.03.04

RANK/RANKL Axis Regulates IL-7-Mediated Membrane-Type Matrix Metalloproteinase MMP14 Expression in Corneal Fibroblasts in GCD2

S. Kim[1], *A. Yeo*[1], *H. Lee*[1,2];
[1]*Institute of Vision Research,Department of Ophthalmology, Yonsei University College of Medicine, seoul, Korea, Republic of,* [2]*Corneal Dystrophy Research Institute, Department of Ophthalmology, Yonsei University College of Medicine, Seoul, Korea, Republic of.*

Granular corneal dystrophy type 2 (GCD2) is caused by a point mutation (R124H) in the transforming growth factor-β (TGF-β)-induced gene (*TGFBI*). The accumulation of the protein TGFBIp in the corneal stroma results in the disruption of corneal transparency and finally in a loss of vision. However, the mechanisms underlying the accumulation of TGFBIp and its clearance are poorly understood. In this study, we show that TGFBI expression was counter-regulated by interleukin-7 (IL-7) in corneal fibroblasts. IL-7 expression was significantly reduced in corneal fibroblasts from patients with GCD2. TGF-β and *TGFBI* expression were reduced upon IL-7 treatment in corneal fibroblasts, suggesting that impaired IL-7 expression in patients with GCD2 affects disease pathogenesis via a failure to control TGF-β expression. Interestingly, the interplay between TGF-β and IL-7 was regulated by the RANKL/RANK signaling cascade. We also found that IL-7 regulates the expression of a membrane-type matrix metalloproteinase (MT-MMP), which plays a crucial role in migration and neovascularization in the cornea via the RANKL/RANK axis. Taken together, we demonstrated that the RANKL/RANK axis regulates TGF-β and *TGFBI* expression via IL-7-mediated MT-MMP regulation in corneal fibroblasts; these findings improve our understanding of the pathogenesis of GCD2.

P.C1.03.05

Mucosal lesions derived from oral cavity identified as lichen planus express high levels of IFN-γ and IL33

M. F. de Carvalho[1], *D. Cavalieri*[2], *S. do Nascimento*[1], *D. V. Ramos*[3], *D. C. Pasqualin*[4], *F. A. Rocha*[1], *D. Heller*[1], ***L. C. Marti***[1];
[1]*Hospital Israelita Albert Einstein - Instituto de Ensino e Pesquisa Albert Einsten, São Paulo, Brazil,* [2]*(2) Centro de Especialidades Odontológicas/Estomatologia – Unidade Alto da Boa Vista - Secretaria Municipal de Saúde de São Paulo, São Paulo, Brazil,* [3]*(3) Instituto de Responsabilidade Social- Programas Governamentais - Hospital Israelita Albert Einstein, São Paulo, Brazil,* [4]*(4) Laboratório de Patologia Clínica - Hospital Israelita Albert Einstein, São Paulo, Brazil.*

Background: Oral lichen planus (OLP) is a chronic inflammatory mucocutaneous disease that is Th1-mediated and affects skin and the oral mucosa with a variety of clinical presentations. Lesions are usually bilateral and are often sensitive or painful. Cutaneous lesions of Lichen Planus (LP) are self-limiting; however, oral lesions are chronic and rarely remissive. Interferon-gamma (IFN-γ) is an important cytokine involved in the regulation of local immune response in OLP, and it has been frequently studied in these lesions. **Aims:** Identify cytokines present in lesions suggestive of OLP and compare them to the controls. The controls were a group of patients with non-specific inflammatory lesions (NSIL). **Methods:** Histopathological image analysis, immunohistochemistry and gene expression were performed in oral mucosal tissue derived from samples suggestive of OLP and controls. All samples were obtained after patient signed an informed consent approved under CAAE number 550537165530010086. **Results:** Our preliminary results demonstrated higher numbers of lymphocytes infiltrating OLP lesions compared to controls (p=0.025) and more T CD4 lymphocytes into epithelial tissue (p=0.006). In addition, the OLP samples showed higher number of apoptotic cells compared to the control (p= 0.047). Regarding cytokine analysis, there were absence or variable expression of IL-17 in both NSIL and OLP groups, significant expression of IFN-γ and IL-33 in OLP group compared to NSIL (p<0.001; p=0.026). **Conclusion:** The lesions characterized as OLP demonstrated higher numbers of apoptotic and inflammatory cells, elevated levels of IFN-γ and IL-33 which described them as more aggressive lesions than NSIL .

P.C1.03.06

Impact of skin T cell secretome on the epidermal barrier during vitiligo

C. Martins[1], *F. Lucchese*[1], *A. Taieb*[1,2], *J. Seneschal*[1,2], *K. Boniface*[1];
[1]*Inserm U1035, BMGIC, Bordeaux University, Bordeaux, France,* [2]*Department of dermatology and Pediatric Dermatology ; National Reference Center of Rare Skin disorders, hôpital Saint-André, Bordeaux, France.*

Introduction: Resident memory T cells have a key role in the development of chronic inflammatory dermatosis, such as vitiligo, the most common depigmenting disorder resulting from the loss of epidermal melanocytes. We previously showed that vitiligo skin is imprinted with resident memory CD8 T cells producing elevated levels of the inflammatory cytokines IFNγ and TNFα, while displaying moderate cytotoxic activity. We further analyzed the cytokine secretion profile of skin T cell secretome and characterized its effect on the inflammatory response and melanocyte alteration. **Materials and Methods:** The cytokine secretion profile of skin T cells was analyzed by Flow cytometry and multiplex ELISA following T cell activation. In addition, primary cultures of normal human epidermal keratinocytes and melanocytes were stimulated in the presence or absence of skin T cell supernatants and expression of genes involved in the inflammatory response, and melanocyte function was assessed by real-time PCR. **Results:** We show that T cell secretome contributes to the development and amplification of the inflammatory response by increasing the expression of chemokines and factors involved in inflammation and T cell homing, such as CXCL9 and CXCL10, by epidermal cells; while inhibiting melanocyte function, leading to the loss of melanocytes. **Conclusion:** Our results highlight the importance of skin T cell secretome in vitiligo pathogenesis, and show the role of T cell soluble factors on both the loss of melanocytes, and on the increase of the inflammatory response through an effect on both keratinocytes and melanocytes.

P.C1.03.07

The effect of the first and third trimester placentas secretory factors obtained from normal pregnancy on the tube-like structure formation by endothelial cells in presence of trophoblast cells

K. L. Belyakova, ***V. A. Mikhailova****, A. R. Sheveleva, E. V. Khokhlova, S. A. Selkov, D. I. Sokolov;*
FSBSI "The Research Institute of Obstetrics, Gynecology and Reproductology named after D.O.Ott", Saint-Petersburg, Russian Federation.

Introduction: Trophoblast cells (TC) interact with endothelial cells (EC) in the uteroplacental contact area. Effects of placenta-derived factors upon local interactions between these cells remain unclear. The aim of the research was to evaluate the influence of placental factors upon formation of tube-like structures by EC in presence of TC. Materials and Methods: EC line EA.Hy926 and TC line JEG-3 were used in the experiment. Placentas were obtained after an elective pregnancy termination of normal 1st-trimester (9-11 weeks, group 1) pregnancy and after caesarean delivery of normal 3rd-trimester (38-39 weeks, group 2) pregnancy. EC and TC were cultured in 24-well-plates pretreated with Matrigel (BD, USA) in the presence of placental conditioned media (CM) with 2,5%FBS for 24 hours (37°C, 4.5%CO_2). Control wells contained DMEM, 2,5%FBS without TC. Then we assessed the number and the length of formed tube-like structures using AxioObserver.Z1 microscope and program AxioVision. Statistical analysis was performed using Mann-Whitney test. Results: In the presence of CM of placentas of both groups we observed an increase in the length and a reduction in the number of tube-like structures formed by EC in the presence of TC, comparing with a spontaneous level of their formation. Also the same parameters in the presence of CM of placentas of group 2 were different from the parameters of group 1. Conclusions: Soluble factors contained in the CM of placentas change the characteristics of the vascular network formed by EC in the presence of TC.
Funding: RSF grant 17-15-01230; State program № AAAA-A18-118011020016-9.

P.C1.03.08

IL-17AF signaling in IMQ-induced psoriasis-like dermatitis and host defense against *Staphylococcus aureus*

S. Moos[1], *T. Regen*[1], *I. Prinz*[2], *C. Reinhardt*[3], *K. Bitschar*[4], *L. Bleul*[5], *B. Schittek*[4], *C. Wolz*[5], *A. Diefenbach*[6], *A. Waisman*[1], *F. C. Kurschus*[1,7];
[1]*Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-Universität Mainz, Mainz, Germany,* [2]*Hannover Medical School, Institute of Immunology, Hannover, Germany,* [3]*CTH, University Medical Center of the Johannes Gutenberg-Universität Mainz, Mainz, Germany,* [4]*Dermatology, University Hospital Tübingen, Tübingen, Germany,* [5]*Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Tübingen, Germany,* [6]*Charité, Berlin, Germany,* [7]*Department of Dermatology, Heidelberg University Hospital, Heidelberg, Germany.*

IL-17A is the signature cytokine of Th17 cells and plays an important role in the development of many autoimmune and inflammatory diseases such as psoriasis and in host defense against microbial organisms. The IL-17 response of Th17 cells and especially of IL-17A-producing γδ T cells is crucial for mice to fight bacterial infections. Among the 6 members of the IL-17 family, IL-17F has the greatest homology to IL-17A. Moreover, IL-17A and IL-17F signal either as homo- or as heterodimers through a dimeric receptor composed of IL-17RA and IL-17RC.
We used IL-17RA full knockout mice or mice with cell type specific deficiencies for IL-17RA to delineate which cell type needs to respond to IL-17 in order to develop Imiquimod-induced dermatitis mimicking human psoriasis. Using this approach, we could clearly define keratinocytes as cell population necessary to respond to IL-17.
Additionally to the mentioned IL-17RA deficient mice, we also analyzed IL-17AF cytokine double knock out mice. We found expanded populations of Rorγt expressing γδ T cells in the IL-17 signaling deficient mice. Furthermore, we could show that IL-17 signaling is important for defense against *Staphylococcus aureus* as the signaling deficient mice developed severe lesions composed of the coccal bacterium.
This work was funded by the DFG (CRC-TR156).

P.C1.03.09

Novel role of complement receptor C5aR2 in uterine natural killer cells during pregnancy

J. Neeb[1], *F. Mey*[1], *J. Figge*[1], *J. Köhl*[1,2], *K. Hoebe*[2], *C. Karsten*[1];
[1]*Institute For Systemic Inflammation Research, Luebeck, Germany,* [2]*Immunobiology, Cincinnati Children's Hospital Medical Center, Cincinnati, United States.*

Uterine natural killer (uNK) cells are known to be important for placental angiogenesis and development during the first trimester of pregnancy. They are the main source for IFNγ and angiogenic factors (VEGF, PLGF). Further, previous studies suggested their role in the pathogenesis of preeclampsia. We have recently shown that peripheral NK (pNK) cells express the complement receptor C5aR2, but not C5aR1. While C5aR1 acts in a pro-inflammatory manner, the function of C5aR2 is still under debate, but hinted to be anti-inflammatory. In this study we hypothesized that C5aR2 expression by uNK cells is indispensable for the development of a healthy gestation. Here, we show potentially a novel role for C5aR2 as a regulator of NK cell functions during pregnancy.
Uteri were removed on gestation day 10 and stained with DBA, CD3, CD122 to analyze NK cell compartment via flow cytometry.
We found that C5aR2 knockout mice have a significantly lower breeding efficiency shown by higher number of resorptions during pregnancy. Further, using C5aR2-tdTomato floxed reporter mice we showed that most of the uNK cells express C5aR2, whereas only subsets of pNK express this receptor. Mechanistically, we were able to show that C5aR2 knockout NK cells produce higher amounts of IFNγ after stimulation in vitro.
All in all, our findings identify a potential novel role for the anaphylatoxin receptor C5aR2 in regulating the NK cell compartment in the uterus and therefore be crucial involved in the outcome of pregnancy.

P.C1.03.10

Cell type-specific complement expression in the retina

D. Pauly[1], *N. Schäfer*[1], *S. Hauck*[2], *A. Grosche*[3];
[1]*Department of Ophthalmology, Regensburg, Germany,* [2]*Research Unit Protein Science, Helmholtz Center Munich, Neuherberg, Germany,* [3]*Department of Physiological Genomics, LMU, Munich, Germany.*

Retinal complement activity has so far been related to macrophages or to the influence of liver-derived complement components. Here we describe for the first time a liver-independent retinal complement system.
Five different cell types were isolated by immunomagnetic separation from murine and human retinae. Expression analysis of specific cell marker, housekeeping and complement component genes was performed by qRT-PCR and Western blot.
All isolated cell populations expressed *c1s*, *c3*, *cfb*, *cfp, cfh* and *cfi*. Strikingly, we distinguished retinal cell types which mainly showed inhibiting complement transcripts from cell populations which synthetized mostly complement activating mRNA. Albino mice showed an age-related change in cell type-specific complement expression levels towards a pro-inflammatory intraretinal milieu. In contrast, retinae with impaired photoreceptor recycling presented only with minimal expression changes. Major changes of local complement expression were observed after induction of a transient ischemia in the mouse retina. A very pronounced upregulation of complement activators was found in several cell types. Complement inhibitor c*fh* was down-regulated in specific cell types, while *cfi* was significantly up-regulated in all investigated cell types.
In conclusion retinal cells produce and regulate complement components independently from the liver-derived complement system in stress situations.

P.C1.03.11

Oxidative stress induces complement-dependent pro-angiogenic and -inflammatory response in retinal pigmented epithelium cells

T. Trakkides, D. Rogalski, N. Schäfer, ***D. Pauly****;*
Department of Ophthalmology, University Hospital, Regensburg, Germany.

Oxidative stress in the aging eye is associated with local inflammatory processes. In this study, we investigated the mechanisms of H_2O_2-induced, complement-dependent inflammatory processes in a human retinal pigment epithelial cell line, ARPE-19.
ARPE-19 viability and integrity were analysed. The expression of components associated with the complement system was determined by qRT-PCR, Western blot, immunofluorescence analyses and secretion of cytokines/ growth factors was measured.
We found that more than 95% of ARPE-19 treated with oxidative stress were but showed disrupted cell-cell junctions. Strikingly, senescent ARPE-19 cells upregulated complement receptors on transcript (*c5ar1*, *cr3*) and protein level (C5aR1) in response to H_2O_2. This gene modulation was associated with priming of the NLRP3 inflammasome, enhanced secretion of pro-angiogenic factors (VEGF, IL-8) and pro-inflammatory cytokine (IL-6). In the late stage of oxidative stress treatment, we observed significant more transcripts of intracellular proteases (*ctsl*, *ctsb*, *serping1*) and of the complement stabilizer (*cfp*) than in untreated controls. In contrast to previously described protective effects of Olaparib in oxidatively stressed retinae, we observed a more pronounced change towards pro-inflammation in Olaparib-treated than in untreated ARPE-19 cells.
Our results show for the first time a functional link between oxidative stress, complement receptors, pro-angiogenic and -inflammatory responses of ARPE-19 cells. These effects suggested an oxidative stress-associated mechanism of C5aR1-regulation in ARPE-19 cells in connection with upregulated intracellular proteases.

P.C1.03.12

Immunomodulatory effect of local complement regulators on retinal pigmented epithelium cells

N. Schäfer[1], *V. Enzmann*[2], ***D. Pauly***[1];
[1]*Department of Ophthalmology, University Hospital, Regensburg, Germany,* [2]*Department of Ophthalmology, University Hospital, Bern, Switzerland.*

Factor H-related 3 (FHR-3) is a complement regulator, which has been associated with retinal degeneration. The function of FHR-3 is so far not completely resolved. Here, we reveal an immunomodulatory effect of FHR-3 on the retinal complement metabolism via the retinal pigment epithelium (RPE).
The expression of complement components, inflammation-associated and metabolic signaling factors was determined in RPE cells following apical stress induced by FHR-3 or FH treatment, respectively. RPE cells were analyzed using qRT-PCR, Western Blots, immunohistology and Multiplex-ELISA.
Our data revealed for the first time a local interaction of FHR-3 with RPE cells. We showed that these cells expressed increased transcripts of *c3, cfb, cr3, nlrp3* and *il-1β* and a reduced mRNA level for *cfh*, *foxp3* and *c3ar1* after FHR-3 treatment. We detected C3, CFB and CR3 proteins in the RPE cells and found a raised secretion of C3, C4 and CFB in supernatants of FHR-3-treated ARPE-19 cells. We detected an increased pro-inflammatory cytokine IL-6 secretion following FHR-3 stress, and an anti-inflammatory IL-18 release of FH-treated RPE cells but not FHR-3 treated cells.
These novel findings reveal a direct influence of the complement regulator FHR-3 on the balance of the ocular immune privilege and indicate FHR-3 as a direct opponent of FH at the blood-retinal barrier.

P.C1.03.13

Absence of STAT1β increases Th1 differentiation and exacerbates concanavalin A-induced hepatitis

A. Puga[1], *K. Meissl*[1], *C. Lassnig*[1,2], *S. Macho-Maschler*[1], *M. Parrini*[1], *D. Hainberger*[3], *W. Ellmeier*[3], *M. Müller*[1,2], *B. Strobl*[1];
[1]*University of Veterinary Medicine Vienna, Vienna, Austria,* [2]*Biomodels Austria, University of Veterinary Medicine Vienna,, Vienna, Austria,* [3]*Institute of Immunology, Medical University of Vienna, Vienna, Austria.*

Signal transducer and activator of transcription 1 (STAT1) is a transcription factor that is crucial for gene regulation in response to all types of interferons (IFN). STAT1 has a crucial role in immunity in both men and mice: loss-of-function of STAT1 leads to a high susceptibility to infections, whereas gain-of-function mutations cause severe autoimmune diseases. Alternative splicing generates two STAT1 isoforms: the full length STAT1α and the truncated STAT1β isoform, which lacks the C-terminal transactivation domain and has compromised transcriptional activities. Using *Stat1*$^{\alpha/\alpha}$ knockin mice (i.e. mice that only express the STAT1α isoform) we show that STAT1β has important regulatory functions in T cells. CD4$^+$ and CD8$^+$ T cells, but not other cell types, from *Stat1*$^{\alpha/\alpha}$ mice have increased levels of STAT1α compared to the levels of both STAT1 isoforms in T cells from wild-type mice. In line with the crucial role of STAT1 in Th1 differentiation, *Stat1*$^{\alpha/\alpha}$ CD4$^+$ T cells had increased levels T-bet and produced considerably more IFNγ upon activation compared to wild-type cells. To investigate whether STAT1β is required to limit excessive IFNγ production *in vivo*, we challenged mice with concanavalin A, an experimental model for autoimmune hepatitis. *Stat1*$^{\alpha/\alpha}$ mice had exacerbated liver damage and higher systemic levels IFNγ compared to wild-type mice. Currently, we investigate whether CD4$^+$ T cells or NKT cells, the main drivers of ConA-induced hepatitis, cause the increased IFNγ levels in *Stat1*$^{\alpha/\alpha}$ mice. In addition, we investigate Th1 cell differentiation during viral infections. Supported by the Austrian Science Fund (FWF, DK-W1212, SFB-F6101and SFB-F6106)

P.C1.03.14

DC subsets and T cells are altered in IPAH patients

D. van Uden*, M. van Nimwegen, T. Koudstaal, P. Heukels, J. A. van Hulst, K. A. Boomars, R. W. Hendriks, M. Kool;*
Department of Pulmonary Medicine, Erasmus MC, Rotterdam, Netherlands.

Pulmonary arterial hypertension (PAH) is a cardiopulmonary disease characterized by high pulmonary arterial pressures, in which idiopathic PAH (IPAH) is the most common. Tertiary lymphoid structures (TLOs) in IPAH lungs indicate a role for immune cell activation. In TLOs, amongst other cell-types, T-cells and dendritic cells (DCs) are present, where DCs are crucial for T-cell activation and TLO maintenance. Thus, we hypothesized that DC subsets and T-cells are altered in IPAH patients.

Therefore, in-depth characterization of DCs and T-cells was performed in peripheral blood mononuclear cells (PBMCs) of IPAH patients and healthy controls (HCs) using 14-color flowcytometry.
The proportion of total conventional DCs (cDCs) was decreased in IPAH patients compared to HCs, in which the percentage of type 1 cDCs (cDC1s) increased and cDC2s was unaltered. Furthermore, decreased percentages of the recently identified T-cell-stimulatory AS-DCs were found in IPAH patients compared to HCs. Strikingly, no differences were observed in memory T-cell proportions, however IPAH T-cells showed reduced capacity to produce interferon (IFN)γ, interleukine (IL)-17 and IL-6.
In conclusion, peripheral DCs and T-cells are altered in IPAH patients, wherein cDCs and AS-DCs are decreased. As cDCs and AS-DCs are potent T-cell stimulators, this could suggest homing towards lung TLOs. Furthermore, the reduced capacity of IPAH T-cells could be a consequence of i) homing towards TLOs or ii) a negative feedback-loop due to a high pro-inflammatory cytokine milieu present in IPAH patients. These data indicate that immune cell activation could play a role in IPAH pathology.
Grant: Dutch Heart foundation (2016T052)

P.C1.03.15
RORγt inhibition selectively targets pathogenic human iNKT and γδ-T cells enriched in Spondyloarthritis while preserving IL-22 responses

K. Venken[1,2], *C. Mortier*[1,2], *M. E. Labadia*[3], *P. Jacques*[1,2], *T. Decruy*[1,2], *J. Coudenys*[1,2], *K. Hoyt*[3], *S. Van Gassen*[1,2], *J. Wahle*[3], *Y. Saeys*[1,2], *G. Nabozny*[3], *D. Elewaut*[1,2];
[1]*Ghent University, Ghent, Belgium,* [2]*VIB Inflammation Research Center, Ghent, Belgium,* [3]*Research and Development Boehringer-Ingelheim, Ridgefield, CT, United States.*

Dysregulated IL-23/IL-17 responses have been linked to inflammatory diseases including psoriasis, psoriatic arthritis and other forms of spondyloarthritides (SpA). IL-23/IL-17 inflammation is controlled by RORγt, the key Thelper17 (Th17) cell transcriptional regulator. RORγt is also expressed by subsets of innate-like T cells, including invariant natural killer T (iNKT) and γδ-T cells, but how this contributes to disorders such as SpA is still unclear. Here we describe a unique population of RORγt^{+}T-bet^{lo}PLZF^{-} iNKT and γδ-hi T cells present in healthy peripheral blood. iNKT and γδ-hi T cells showed profound IL-23 mediated Th17-like immune responses and were clearly enriched within inflamed joints. Moreover, selective depletion of iNKT and γδ-Tcells in synovial fluid mononuclear cell samples led to a significant decrease (>70%) in IL-17 responses underscoring the pathogenic features of these cells in SpA. Interestingly, unsupervised clustering analyses by FlowSOM iterations revealed a marked heterogeneity of human blood iNKT and γδ-T cells which seemed skewed in SpA patients. Strikingly, RORγt inhibition blocked γδ17 and iNKT17 cell function while selectively sparing IL-22^{+} subsets. Overall, these findings highlight a unique diversity of human RORγt^{+} T cells and underscore the potential of RORγt antagonism to modulate aberrant type 17 responses.

P.C1.03.16
IgE-Bet v 1 oligomer complexes induce specific T cell activation via CD23 mediated facilitated antigen presentation more efficiently than IgE-Bet v 1 monomer complexes

S. Villazala[1], *G. Hofer*[2], *P. Gattinger*[3], *H. Breiteneder*[3], *S. Vrtala*[3], *C. Lupinek*[3], *W. F. Pickl*[4], *W. Keller*[2], *R. Valenta*[3], *V. Niederberger*[1], *J. Eckl-Dorna*[1];
[1]*Medical University of Vienna, Department of Otorhinolaryngology, Vienna, Austria,* [2]*University of Graz, Institute for Molecular Biosciences, Graz, Austria,* [3]*Medical University of Vienna, Department of Pathophysiology and Allergy Research, Vienna, Austria,* [4]*Medical University of Vienna, Department of Cellular Immnunology and Immunohematology, Vienna, Austria.*

CD23, the low affinity receptor for IgE, mediates facilitated antigen presentation (FAP). FAP, a process known to perpetuate and amplify pathological T cell responses in allergy, is initiated by the binding of IgE-allergen complexes to CD23. Those complexes are endocytosed and peptides from those antigens are displayed on MHC II to be recognised by specific T cells. Our aim was to study the effects of different types of IgE-Bet v 1 complexes on FAP. Epstein Barr virus transformed B cells were incubated with different concentrations and ratios of chimeric Bet v 1 specific Bip 1 IgE (CB1 IgE) and recombinant Bet v 1 in monomeric or oligomeric form.
Primed cells were incubated with Jurkat T cells, which expressed a T cell receptor (TCR) specific for Bet v 1 and a luciferase reporter gene under the control of IL2 promotor. Equimolar concentrations of CB1 IgE-oligomer Bet v 1 complexes induced higher specific T cell activation when compared to CB1 IgE-Bet v 1 monomer complexes, independently of their ratio. Likewise 25-125 fold lower concentrations of CB1 IgE-Bet v 1 oligomer than CB1 IgE-Bet v 1 monomer complexes were sufficient to induce specific T cell activation. Blocking of CD23 using anti-CD23 antibody completely abrogated specific T cell activation triggered by IgE-Bet v 1 complexes. In conclusion, IgE-Bet v 1 oligomer complexes provoke specific T cell activation more efficiently than IgE-Bet v 1 monomer complexes. These data indicate that the oligomerisation state of IgE-allergen complexes greatly affects FAP and subsequent allergen-specific T cell activation.

P.C1.03.17
Foxp3^{+} RORγT^{+} regulatory T cells depend on CD27/CD70 costimulation

I. Vogel, *V. Acolty, M. Moser;*
Laboratory of Immunobiology, IBMM, Université Libre de Bruxelles (ULB), Gosselies, Belgium.

CD27/CD70 costimulation enhances T-cell survival, expansion and effector function. However, in Th17 cells, which express the transcription factor RORγT, CD27 signalling specifically suppresses the production of the proinflammatory cytokine IL-17. Consequently, mice lacking CD27 or its ligand CD70 have a higher number of IL-17 producing RORγT^{+} Th17 cells compared to WT mice and display exacerbated experimental autoimmune encephalomyelitis (EAE). This suggests a tight regulation of the effector function of RORγT expressing cells by CD27/CD70 signalling.
Recently, so called T-regulatory-17 (Tr17) cells have been described which express the transcription factors Foxp3 and RORγT and specifically suppress Th17 cells during EAE. To examine if Tr17 cells are equally dependent on CD27 costimulation, we induced EAE in CD27 and CD70 knock-out (KO) mice. While KO mice have overall less Foxp3^{+} Treg cells in a steady state, their number rapidly increases in response to immunization to the level of WT mice. Tr17 cells were present in the peripheral lymph nodes of both WT and KO mice. However, we found a significant reduction of this Treg subset in the central nervous system (CNS) of mice lacking CD27 or CD70. Tr17 cells in the periphery of KO mice displayed a more active phenotype (CTLA-4^{high}, CD103^{high}) compared to WT mice suggesting that activated cells are trapped in the lymph nodes and unable to migrate to the CNS.
Transcriptomic (single-cell) profiling and functional analysis of Tr17 cells in KO *vs.* WT mice will further elucidate the role of CD27/CD70 signalling in RORγT^{+} Treg cells and discover downstream signals.

P.C1.03.18
The role of S100A8 and S100A9 in murine keratinocytes

C. Weise[1], *J. Latzel*[1], *K. Loser*[2], *J. Roth*[1];
[1]*Institute of Immunology, Münster, Germany,* [2]*Department of Dermatology, Münster, Germany.*

Introduction: S100A8 and S100A9, also known as myeloid-related protein-8 (MRP8) and MRP14, are damage-associated molecular pattern molecules (DAMPs). Both proteins are highly up-regulated in autoimmune diseases of human skin like psoriasis as well as in certain *in vivo* models (e.g. Imiquimod induced psoriasis like phenotype).
Methods: Keratinocytes collected from adult tail skin of either wildtype or S100A9-/- mice were differentiated under high calcium conditions (1 mM CaCl2). Furthermore keratinocytes of both mice strains were stimulated with different inflammatory mediators. Cells were harvested at different time points and analysis of gene or protein expression was performed.
Results: Both, wildtype and S100A9-/- cells, show a typical expression pattern of genes responsible for differentiation. During differentiation an increased gene expression of both S100 proteins could be detected in wildtype but not in S100A9-/- (here only S100A8) cells. On protein level S100A9-/- cells show no expression of S100A8 and S100A9 whereas wildtype cells express both proteins in increasing amounts during differentiation. Wildtype keratinocytes stimulated with IL-1α, IL-17A, IL-17F, TNFα and Flagellin show a significant elevation of S100A8 and S100A9 on mRNA and protein level compared to unstimulated or IL-13 treated cells.
Conclusion: Both wildtype and S100A9-/- keratinocytes show the same gene expression pattern of proteins known for differentiation, indicating no direct correlation of S100 proteins and keratinocyte differentiation. Surprisingly S100A9-/- keratinocytes do not express S100A8 although mRNA level were increasing during differentiation and stimulation with inflammatory mediators. These results indicate an inflammation and differentiation dependent mechanism of S100 protein expression.

P.C1.03.19
Thymus-derived Foxp3+ regulatory T cells upregulate RORgt expression under inflammatory conditions

J. YANG[1], *M. Zou*[1,2], *J. Pezoldt*[1], *X. Zhou*[2], *J. Huehn*[1];
[1]*Helmholtz Centre for Infection Research, Braunschweig, Germany,* [2]*Key Laboratory of Pathogenic Microbiology and Immunology, University of the Chinese Academy of Sciences, Beijing, China.*

Foxp3^{+} regulatory T cells (Tregs) co-expressing the Th17-lineage specification factor RORγt represent a unique Treg subpopulation that has been reported to be induced upon response to gut microbiota within the intestinal immune system. Hence, RORγt^{+} Tregs are considered to solely consist of peripherally-induced Foxp3^{+} Tregs (pTregs), and the possibility that also thymus-derived Treg (tTregs) can upregulate RORγt expression and contribute to the pool of RORγt^{+} Tregs was largely ignored. Here, we expand our knowledge on the origin of RORγt^{+} Tregs by demonstrating that also tTregs can attain RORγt expression. In transgenic Foxp3$^{\Delta CNS1Cre}$ reporter mice, a substantial fraction of CNS1-independent Tregs, predominantly consisting of tTregs, was found to coexpress RORγt. In additon, genuine tTregs isolated from thymi of Foxp3^{hCD2}RAG^{GFP} reporter mice initiated RORγt expression both *in vitro* and *in vivo*, particularly under inflammatory conditions. In conclusion, our data demonstrate that tTregs can upregulate RORγt expression under inflammatory conditions and that hence RORγt^{+} Tregs can be regarded as a heterogeneous population consisting of both pTregs and tTregs.

P.C1.03.20
Evolution of CD4+ T cell immunity to viral infection: analysis of systemic and local responses

***B. J. Meckiff**, H. M. Long;*
Institute of Immunology and Immunotherapy, Birmingham, United Kingdom.

BACKGROUND: Most studies investigating the immune response to viral infection have focussed on systemic immunity. However, mounting evidence suggests that immunity also involves localised non-circulating resident memory populations retained in specific anatomic compartments. To date, there is limited knowledge of resident memory $CD4^+$ T cells induced by natural virus infection in humans.
Here we compare systemic $CD4^+$ T cell immunity against the common herpesvirus Epstein-Barr virus (EBV) with the immune response at the site of infection, the tonsils. Clinical identification of Infectious Mononucleosis (IM) patients undergoing primary infection has enabled investigations of acute primary infection through to persistent carriage. A greater understanding of resident T cell memory in humans is paramount for the development of vaccine strategies.
METHODS: Matched PBMCs and tonsillar UMs were collected from patients suffering from acute IM or from healthy EBV carriers undergoing tonsillectomy. We used EBV-MHCII tetramers, representing a range of viral $CD4^+$ T cell epitopes, to investigate the frequency and phenotype of virus-specific $CD4^+$ T cells in both compartments.
RESULTS: Primary infection induces expanded populations of activated virus-specific $CD4^+$ T cells in the blood of IM patients, but only low frequencies are detected in the tonsils. Following resolution of IM symptoms, EBV-specific $CD4^+$ T cells are maintained in memory at a low frequency in both the circulation and at the site of infection. Furthermore, in long-term virus carriage, some virus-specific $CD4^+$ T cells in the tonsil express resident memory-associated markers. Therefore EBV infection induces a population of virus-specific $CD4^+$ T cells that are retained within the tonsil, with a different phenotype to circulating cells.

P.C1.04 Maintenance and local regulation of tissue specific immunity - Part 4

P.C1.04.01
Kidney disease alters the intestinal microbiota and regulates intestinal inflammation

***K. Aono**, K. Nishiyama, H. Nakajima, T. Takeuchi, Y. Azuma;*
Laboratory of Veterinary Pharmacology, Osaka Prefecture University Graduate School of Life and Environmental Science, Izumisano, Japan.

A number of organ-organ crosstalk is involved in homeostasis. Considering gastrointestinal symptoms are common in patients with renal failure, the aim of this study was to elucidate the relationship between gastrointestinal motility and gastrointestinal symptoms in chronic kidney disease. We performed studies with C57BL/6 mice with chronic kidney disease following 5/6 nephrectomy. Gastrointestinal motility was evaluated with ex vivo responses of ileum and distal colon strips to electrical field stimulation. Feces were collected from mice and the composition of the gut microbiota was analyzed by 16S rRNA sequencing. Mice with chronic kidney disease after 5/6 nephrectomy showed the decreased number of fecal and this constipation is correlated with the suppressed contraction response in the ileum motility and decreased relaxation responses in the distal colon motility. Spermine, one of the uremic toxins, inhibited the contraction response in the ileum motility, but 4 kind of uremic toxins showed no effect on the relaxation responses in the distal colon motility. 5/6 Nephrectomy mice had the disturbed balance of gut microbiota. The motility dysregulation and constipation were cancelled by antibiotic treatments. Expression levels of IL-6, TNF-alpha, and iNOS in 5/6 nephrectomy mice were increased in the distal colon but not in the ileum. In addition, macrophage infiltration in 5/6 nephrectomy mice were increased in the distal colon but not in the ileum. We found 5/6 nephrectomy to alter the gastrointestinal motility and cause constipation by changing the gut microbiota and colonic inflammation. These findings indicate that renal failure was remarkably associated with gastrointestinal dysregulation.

P.C1.04.02
Interleukin-19 protects Th2-mediated IBD model mice

***Y. Azuma**, T. Takeuchi;*
Laboratory of Veterinary Pharmacology, Osaka Prefecture University Graduate School of Life and Environmental Science, Izumisano, Japan.

IL-19 was originally found by sequence homology to IL-10, and is a member of the IL-10 family. However, little is known about the exact immunological role of IL-19 in the regulation of inflammatory bowel disease. Ulcerative colitis (UC) involves the superficial mucosal and submucosal layers of the colon and is driven by Th2 cytokines, such as IL-4, IL-5, and IL-13. In this study, we investigated the role of IL-19 in oxazolone-induced colitis which is useful for the study of the Th2 inflammatory response and resembles the symptom seen in UC. Balb/c genetic background IL-19 knockout mice (KO) were used. To presensitize mice, 150 µL of a 4% oxazolone in 100% ethanol was applied in the shaved abdominal skin. Seven days after presensitization, colitis was induced by intrarectal administration with 100 µL of 3% of oxazolone in 50% ethanol using a plastic catheter. The colitis was evaluated by analyzing body weight loss and histology of the distal colon. IL-19KO showed severe weight loss. Histological analysis revealed that the distal colon in IL-19KO exhibited increased numbers of infiltrating cells and a general loss of tissue architecture. In IL-19KO, oxazolone treatment increased colonic MPO activity. Serum IgE levels and the number of circulating eosinophil were significantly elevated in IL-19KO. CD4-positive T-cells isolated from lymph node of IL-19KO produced elevated amounts of IL-4 and IL-9, but not IL-5 and IL-13. IL-19 plays an anti-inflammatory role in the Th2-mediated colitis model, suggesting that IL-19 may represent potential therapeutic target for reducing colonic inflammation.

P.C1.04.03
Anti-IL-17A therapy resolves skin lesions and vascular dysfunction in mouse models of psoriasis

***A. Brand**[1], R. Schüler[1,2], C. Wohn[3], P. Wenzel[2], A. Waisman[1], S. Karbach[1,2], B. E. Clausen[1];*
[1]Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-University Mainz, 55131 Mainz, Germany, [2]Center for Thrombosis and Hemostasis, University Medical Center of the Johannes Gutenberg-University Mainz, 55131 Mainz, Germany, [3]Department of Immunology, Erasmus MC, 3015 GE Rotterdam, Netherlands.

Introduction: Besides skin inflammation, patients with severe psoriasis suffer from an increased risk of cardiovascular mortality. We hypothesized that Interleukin (IL)-17A is a key cytokine connecting the pathogenesis of psoriasis to the development of cardiovascular disease. Indeed, we previously demonstrated that high-level overexpression of IL-17A in keratinocytes leads to severe psoriasis-like skin disease and drives vascular inflammation, endothelial dysfunction and arterial hypertension in mice.
Methods: To further determine the role of IL-17A in the correlation of psoriatic skin inflammation and cardiovascular disease, we targeted IL-17A expression to all $CD11c^+$ myeloid cells and assessed the development of psoriatic skin lesions and vascular dysfunction in these CD11c-$IL17A^{ind}$ mice. In addition, we investigated whether neutralization of IL-17A can protect CD11c-$IL17A^{ind}$ mice from the concurrent development of skin lesions and cardiovascular disease.
Results: Constitutive low-level expression of IL-17A by $CD11c^+$ cells resulted in the gradual development of chronic skin lesions, resembling macroscopic, histologic and genetic hallmarks of psoriatic plaques. The onset and incidence but not the severity of cutaneous disease was IL-17A dose dependent. Similarly, vascular dysfunction correlated with peripheral IL-17A levels. Notably, successful anti-IL-17A treatment of psoriatic skin lesions diminished peripheral oxidative stress levels, pro-inflammatory cytokines and vascular inflammation.
Conclusion: These data highlight the pivotal role of IL-17A linking plaque formation and vascular disease in psoriasis. Moreover, neutralization of IL-17A can rescue both the skin and cardiovascular phenotype. Hence, CD11c-$IL17A^{ind}$ mice represent a valuable tool to investigate the effects of biologics on chronic skin and cardiovascular disease in psoriasis.

P.C1.04.04
PD-1-dependent dysregulation of type-2 innate lymphoid cells in a murine model of obesity

*G. Oldenhove[1], E. Boucquey[1], A. Taquin[1], V. Acolty[1], L. Bonetti[1], B. Ryffel[2], M. Le Bert[2], **K. Englebert**[1], L. Boon[3], M. Moser[1];*
[1]IBMM, Department of Molecular Biology, Université Libre de Bruxelles, Gosselies, Belgium, [2]INEM, UMR7355, Molecular Immunology, University and CNRS, Orléans, France, [3]Bioceros, Yalelaan 46, 3584 CM, Utrecht, Netherlands.

Recent observations have clearly highlighted the critical role of type 2 innate lymphoid cells in maintaining the homeostasis of adipose tissues in humans and mice. This cell population promotes beiging and limits adiposity directly and indirectly by sustaining a Th2-prone environment enriched in eosinophils and alternatively activated macrophages. Accordingly, the number and function of ILC2s are strongly impaired in obese individuals. In this work, we sought to determine the factor(s) leading to ILC2 destabilization upon high fat feeding. We identified the PD-1/PD-L1 pathway as a negative checkpoint of ILC2 homeostasis and function. TNF appears to play a central role, triggering IL-33-dependent PD-1 expression on ILC2s and recruiting/activating PD-$L1^{hi}$ M1 macrophages. PD-1 blockade partially restored the type 2 innate axis, raising a possibility to restore tissue homeostasis.

P.C1.04.05

A novel humanized mouse model to study skin immunology

*M. M. Klicznik[1], **L. M. Gail**[1], R. Holly[1], A. Benedetti[1], A. Sir[2], R. Reitsamer[2], E. M. Murauer[3], D. J. Campbell[4], I. K. Gratz[1,3];*
[1]University of Salzburg, Department of Biosciences, Salzburg, Austria, [2]Breast Center University Hospital Salzburg, Paracelsus Medical University Salzburg, Salzburg, Austria, [3]Division of Molecular Dermatology and EB House, Salzburg, Austria, [4]Benaroya Research Institute, Seattle, United States.

Human skin is a complex barrier organ with elaborate immune functions to prevent infection by pathogens and reactions against harmless antigens. Numerous immune cells reside in human skin and communicate with each other as well as with the skin tissue cells (e.g. epidermal keratinocytes) in order to mediate appropriate immune responses. We hypothesize that these interactions are crucial to maintain homeostasis within the skin and aim to investigate them. Because murine and human skin differ in structure and cell composition we established a skin-humanized xeno-graft mouse model to investigate immune mechanisms in vivo. In the model we generate a simplified skin tissue consisting of fibroblasts and keratinocytes only, thus featuring a minimal organotypic skin tissue. This can be reconstituted with different immune cell types, allowing for the study of e.g. T cell-APC, T cell-keratinocyte or T-cell-microbe cross-talk. We can successfully follow T cell infiltration of the skin, started to characterize local T cell - APC interactions and found that these lead to improved T cell infiltration of the skin and local T cell proliferation in the presence of a microbial antigen. Further on we will use this model to study the mechanisms of maintenance and recruitment of the numerous skin T cells and their immunological and tissue regenerative function. M.M.K. is supported by a DOC-fellowship from the Austrian Academy of Science. The project was supported by a Debra International Grant awarded to E.M.M. and I.K.G. and a grant from the National Institute of Health (R01AI127726) awarded to D.J.C. and I.K.G..

P.C1.04.06

Hallmark features of atopic skin disease affect keratinocyte-derivedexosomal compartment

***L. Hovhannisyan**[1], A. Łuczak[1], A. Kobiela[1], X. Wang[2,3], H. Sheldon[4], E. Giannoulatou[5], S. Taylor[5], G. Ogg[2], D. Gutowska-Owsiak[1,2];*
[1]Institute of Biotechnology UG, Intercollegiate Faculty of Biotechnology of University of Gdańsk and Medical University of Gdańsk, Gdańsk, Poland, [2]MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom, [3]State Key Laboratory of Military Stomatology, Department of Oral Medicine, School of Stomatology, The Fourth Military Medical University, Xi'an Shaanxi Province, China, [4]Cancer Research UK Molecular Oncology Laboratories, Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom, [5]Computational Biology Research Group, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom.

INTRODUCTION: Atopic dermatitis (AD) is a common skin disease combining skin barrier dysfunction and aberrant epidermal differentiation with inflammation. Recent milestone discoveries highlighted the importance of a keratinocyte protein, filaggrin, linking insufficiency in epidermal barrier with inflammatory state and induction of allergic phenotype resulting in sensitisation to allergens.
Exosomes are secreted vesicles acting as messengers between cells; they have functional impact on recipient cells by delivery of a protein, lipid or nucleic acid cargo. Published data suggest changes in the secretion of vesicles upon cellular maturation, differentiation and exposure to cytokines. Here, we investigated whether the two hallmarks of allergic skin disease, i.e. keratinocyte differentiation/epidermal barrier dysfunction and inflammatory *milieu* induce changes in keratinocyte exosomal compartment.
METHODS: We used filaggrin knock-down keratinocytes and allergic mediators (histamine; IL-4/IL-13; IL-17A and IL-22) followed by mRNA profiling and enrichment analysis (FunRich tool) to observe changes in keratinocyte-derived exosomal compartment. We isolated exosomes from conditioned keratinocyte medium (by ultracentrifugation) and analysed exosomal output by WB with the use of common exosomal markers.
RESULTS: Expression of genes encoding exosome-enriched proteins was altered in both filaggrin-insufficient keratinocytes and keratinocytes exposed to inflammatory mediators enriched in atopic skin. Western blot analysis confirmed the impact of keratinocyte differentiation state and stimulation on exosome production.
CONCLUSIONS: Hallmarks of atopic skin disease, i.e. inflammation and barrier dysfunction affect quantitatively and qualitatively keratinocyte-derived exosomal pool, suggesting changes in inter-cellular communication across skin barrier. These are likely to contribute to pathogenesis of AD. The mechanisms remain largely elusive and warrant further study.

P.C1.04.07

Telomeres of T cells as a marker of type 1 diabetes progression in children

***D. Iwaszkiewicz-Grzes**[1], M. Gliwinski[1], A. Woloszyn-Durkiewicz[2], J. Sakowska[1], M. Żalińska[2], M. Hennig[2], M. Myśliwiec[2], P. Trzonkowski[1];*
[1]Department of Clinical Immunology and Transplantology, Medical University of Gdansk, Gdansk, Poland, [2]Department of Pediatric Diabetology and Endocrinology, Medical University of Gdansk, Gdansk, Poland.

Introduction: Type 1 diabetes (T1D) is a chronic disease caused by autoimmune destruction of beta-cells in pancreatic islets, which is followed by hyperglycaemia and clinical symptoms. Genetical and environmental factors play an important role in pathogenesis of T1D. Chronic inflammation associated with autoimmune diseases influences the immune system affecting the replication of lymphocytes. It has been observed that in relation to chronic inflammation, immune cells proliferate more intensively, which causes acceleration of cell-aging. Cellular age can be estimated by measuring the telomeres length. In somatic cells, telomeres get shorter with every cell division and as a result, they are the indicator of the cellular senescence.
Materials and Methods: Samples from were collected from 29 patients with newly diagnosed and 25 patients with long-standing type 1 diabetes. CD4+ and CD8+ T cells were isolated from fresh peripheral blood mononuclear cells. We measured telomeric sequences in vertebrate interphase hematopoietic cells. We used MOLT-4 cells as a reference.
Results: There was an association between age, time of diagnose and telomere length in CD4+ and CD8+ T cells in children, which was especially evident in CD4+ T cells.
Conclusions: The duration of the disease influences the length of telomeres in lymphocytes.
This work has been supported by National Centre for Research and Development, Poland: LIDER/160/L-6/14/NCBR/2015 and STRATEGMED1/233368/1/NCBR/2014.

P.C1.04.08

The effect of progesterone on MMP7 and MMP13 expression in mouse model of systemic sclerosis

***e. izady**[1], F. Vafashoar[1], M. Assarehzadegan[1], H. Poormoghim[2], j. Kuhpayehzadeh[3], N. Mojtabavi[4];*
[1]Immunology department,Iran university medical science, Tehran, Iran, Islamic Republic of, [2]Scleroderma Study group, Firuzgar Hospital,Iran University of medical sciences,, Tehran, Iran, Islamic Republic of, [3]Community Medicine and Public health research center, Iran University of medical sciences, Tehran, Iran, Islamic Republic of, [4]Immunology department, Iran University of medical sciences,Iran university medical science, Tehran, Iran, Islamic Republic of.

Background: Gender medicine is a new era of science which focuses on the impact of sex hormones and gender on normal physiology, pathobiology and clinical features of diseases. In our previous study we showed that supra physiological dose of progesterone exacerbate the lung fibrosis in a mouse model of scleroderma. Matrix metalloproteinases are a group of enzymes which play a role in tissue remodeling and fibrosis. Whereas the abnormal expression of MMP2 and MMP9 are indicated in the pathogenesis of systemic sclerosis, fewer studies are done on MMP13 and MMP7 which are expressed by epithelial cells, fibroblasts and macrophages of the lungs. They are involved in the pathogenesis of COPD, IPF and different lung diseases therefore we aimed to investigate the effect of progesterone on the expression of these two enzymes in lungs of mouse model of scleroderma.
Method: Female mice received progesterone for 28 and 21 days in addition to 28 days bleomycin. On day 29 mice were sacrificed and the expressions of these two enzymes in lungs were analyzed by real time PCR.
Result: We found that bleomycin significantly downregulated the expression of MMP7 and MMP13 and co-administration of progesterone and bleomycin declined the expression of these enzymes but not in a significant manner.
Conclusion: While progesterone cannot reduce the expression of MMP7 and MMP13 in a mouse model of lung fibrosis more investigation on other player of fibrosis are necessary.
Keywords: Systemic sclerosis, Progesterone, Fibrosis, Bleomycin, MMP7, MMP13

P.C1.04.09

IL-36 signalling modulates CD4$^+$ T cell responses involved in the pathogenesis of IBD

***G. Leon**[1,2], S. O'Neill[1], M. Gogarty[1,2], S. Hussey[1], P. T. Walsh[1,2];*
[1]National Children's Research Centre, Crumlin, Dublin, Ireland, [2]Dept of Clinical Medicine, Trinity Translational Medicine Institute, Trinity College Dublin, Dublin, Ireland.

Despite significant therapeutic advances the global incidence of Inflammatory Bowel Diseases (IBD) continues to rise. Aberrant lymphocyte and immune cell activation and infiltration into the colonic mucosa of patients are characteristic of inflammation in IBD and has been demonstrated to occur due to inappropriate innate signalling.
In this study we demonstrate increased levels of the pro-inflammatory innate cytokine IL-36α in the serum of paediatric patients with IBD. Importantly, the elevated levels of IL-36α observed are negatively correlated with expression of IL-36Ra, indicating an environment permissive to enhanced IL-36 signaling among these patients. Pathogenic autoimmune T cell responses play a pivotal role in the pathogenesis of IBD and we demonstrate that IL-36 exerts potent effects on specific CD4+ T cell lineages implicated in disease. Specifically, IL-36α directly enhances murine naïve CD4+ Thelper cell (T_H), T_H1, T_H2 and T_H9 effector lineage responses, independently of potent IL-2 induction during early T_H cell activation. In contrast, IL-36α directly inhibits the differentiation of non-pathogenic T_H17 and iTreg cells. Critically, the absence of the IL-36 receptor, specifically on CD4+ T cells, results in altered T cell activation *in vivo* while conferring significant protection from disease progression in a T cell driven model of colitis.
These data indicate an important role for IL-36 family members in T_H cell-mediated inflammation in the context of IBD, thus rendering these cytokines attractive targets for therapeutic intervention.

P.C1.04.10
The role of IL-19 in CCl_4-induced liver fibrosis

***M. Miki**, H. Nakajima, T. Takeuchi, Y. Azuma;*
Laboratory of Veterinary Pharmacology, Osaka Prefecture University Graduate School of Life and Environmental Science, Izumisano, Japan.

IL-19 is a member of the IL-10 family and is an anti-inflammatory cytokine produced mainly by macrophages. Liver fibrosis results from chronic liver injury-mediated inflammation and activation of hepatic stellate cells. However, the involvement of IL-19 in liver fibrosis is not yet fully understood. We investigated the immunological role of IL-19 in carbon tetrachloride (CCl_4)-induced liver fibrosis model mice. Balb/c genetic background IL-19 knockout (KO) mice and age-matched wild-type (WT) mice were used. Liver fibrosis was induced by CCl_4 injection (0.2 mL of 50% solution/kg, 2 times per week) for 8 weeks. Histological evaluation in the liver was assessed by HE staining, Azan staining, and α-SMA immunohistochemistry. mRNA expression in the liver was analyzed by quantitative real-time PCR. In CCl_4-induced liver fibrosis, serum analysis revealed that level of ALT was decreased in L-19 KO mice compared with WT mice. IL-19 KO mice presented exacerbated fibrosis by the morphometric assessment of the total area positively stained with Azan. Moreover, α-SMA expression were increased in liver sections of IL-19 KO compared with WT mice. Additionally, mRNA expression levels of TGF-β and α-SMA were increased in IL-19 KO mice compared with WT mice. These findings indicate that IL-19 has previously undocumented roles in the progress of fibrosis. Enhancement of IL-19 signaling pathway may present therapeutic treatments of liver fibrosis.

P.C1.04.11
Biomarkers in Immune response of fungi inoculated mice

***A. J. Odebode**[1], A. Adekunle[2], E. Farombi[3];*
[1]Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria, [2]Department of Botany, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria, [3]Faculty of Basic Medical Science, University of Medicine, Ibadan., Ibadan., Nigeria.

Fungi are an increasing public health problem worldwide because they have a great impact on human health. A mouse model was devised to compare the adverse effects provoked by four most abundant fungi isolated from southwest, Nigeria.Sixty Balb/b albino mice were grouped into nine treatments of six per cage with a control group. The animals were exposed intranasally to the spores of *A. flavus, A. penicilloides, Penicillium citrinum* and *Penicillium chrysogenum* at 2.3×10^7 and 3.2×10^5 m/L for 24hours. Both dose-response, time-course inflammatory and toxic responses were investigated after a single dose of the microbes. The spores of *A. flavus,* and *A. penicilloides* provoked a very intense acute inflammation indicated by production of increased malondialdehyde, myeloperoxidase, protein in the lungs. The inflammatory cell response in the lungs was more severe and varies with each organism. White blood cells were significantly higher in *Penicillium funiculosum*, *Aspergillus terreus* and *P. chrysogenum* inoculated mice compared to control. All the fungi inoculated resulted in significant ($p<0.05$) increase in the monocyte and basophils cell. *P. citrinum* significantly increased the eosinophils as *A. terreus* increased the lymphocytes. The neutrophils content was higher in the *A. penicillioides, P. chrysogenum* and *P. citrinum* incoculated treatments. Both haemoglobin and red blood cell count were significantly increased in the mice inoculated with *A. funiculosum*, *P. chrysogenum* and *A. flavus* compared to control group. The results show that the selected microbes have potential to cause inflammatory and toxic responses after airway exposure in mice.

P.C1.04.12
Cutaneous drug eruptions

***L. Pajaziti**, S. Sopjani, A. Krasniqi, O. Vogel, A. Pajaziti;*
University Clinical Center of Kosovo, Prishtina, Kosovo, Republic of.

Drug reactions are common. Their frequency increases with the increased use of drugs. The intensity of the reactions is different and ranges from mild forms that are more frequent to life-threatening. Clinical manifestations of these reactions are different. The aim of this study is to explore some of the characteristics of the drug reactions in our hospitalized patients: the most common clinical forms of the cutaneous drug eruptions, the identification of the causative drugs, circumstances, and the possible correlation of a drug with a given clinical type. In this study, 128 patients (58 women and 70 males) with cutaneous drug eruption were included.
Six types of reaction were observed: erythema multiforme / Steven-Johnson syndrome, urticaria, exanthematous drug eruption, fixed drug eruption, erythema nodosum, and angioedema. Of them, the dominant pattern was erythema multiforme (34.37%), followed by urticaria (31.25%), and exanthematous reactions (19.53%). Nonsteroidal anti-inflammatory drugs and acetaminophen were the most common cause of drug reactions, followed by antibiotics (39.06%). A drug may cause some clinical patterns of reaction. Uncontrolled use of nonsteroidal anti-inflammatory drugs increases the likelihood of drug eruptions.

P.C1.04.13
IL-35 maintains regulatory T cells phenotype to suppress diabetic nephropathy

*E. Eriksson[1], Z. Luo[2], S. Varli[1], M. Mejia-Cordova[1], D. Espes[1], L. Thorvaldson[1], M. Blixt[1], P. Carlsson[1], P. Hansell[1], S. Sandler[1], **K. Singh**[3];*
[1]Dept of Medical Cell Biology, Uppsala University, Uppsala, Sweden, [2]Dept. of Medical Cell Biology, Uppsala University, Uppsala, Sweden, [3]Uppsala University, Uppsala, Sweden.

Diabetes causes an elevation of the blood glucose level and a long-term hyperglycemia that contributes to kidney damage, i.e. diabetic nephropathy (DN). DN exhibits signs of inflammation and kidney infiltration of mononuclear-cells. RegulatoryT-cells (Treg-cells) maintain the homeostasis of the immune system, specifically by producing anti-inflammatory cytokine IL-35. Despite intensive research the role of both Treg-cells and IL-35 is not yet clear. Herein, we determined the proportions of Treg-cells, IL-35$^+$Treg-cells and IL-17a$^+$Treg-cells in type 1 diabetes (T1D) patients with or with out DN, and matched them for age, sex and body mass index with healthy controls (HC). Treg-cells and IL-35$^+$Treg-cells was decreased in DN and T1D patients, whereas IL-17a$^+$Treg-cells were increased compared to HC. To further advocate the role of Treg-cells in DN of Treg cells in the kidneys of multiple low dose streptozotocin (MLDSTZ) treated mice and in NOD female mice were determined. We found an increase in the numbers of Treg-cells and an infiltration of mononuclear-cells in the kidneys of MLDSTZ, and NOD mice. Thus, our data reveal that Treg-cells are increased in kidneys of mouse models of T1D. However, the upregulation of Treg-cells did not protect against both hyperglycemia and infiltration of mononuclear-cells in kidneys. In addition, IL-35 treatment prevented the mononuclear cell infiltration and maintained the phenotype of Treg-cells in kidneys of MLDSTZ and NOD diabetic mice, and albumin creatinine ratio was lowered in IL-35 treated mice compared to vehicle mice, suggesting that IL-35 could be investigated to prevent mononuclear-cell infiltration in kidneys and perhaps in DN.

P.C1.04.14
The role of interleukin-19 in NAFLD/NASH mice model

***Y. Ushikai**, K. Hirota, H. Nkajima, T. Takeuchi, Y. Azuma;*
Laboratory of Veterinary Pharmacology, Osaka Prefecture University Graduate School of Life and Environmental Science, Izumisano, Japan.

IL-19 is a member of the IL-10 family and is an anti-inflammatory cytokine produced mainly by macrophages. Nonalcoholic fatty liver disease (NAFLD) is highly associated with the metabolic syndrome, and occurs as a more serious form of the disease, nonalcoholic steatohepatitis (NASH). NASH is diagnosed pathologically by histological evaluation of fibrosis, inflammation, and other features, such as hepatocyte ballooning. However, the involvement of IL-19 in liver inflammation and liver fibrosis is not well understood. We investigated the immunological role of IL-19 in diet-induced NAFLD/NASH model mice. IL-19 knockout mice (KO) and wild-type mice (WT) were fed a choline-deficient and high-fat diet (60kcal% fat) with 0.1% methionine and 2% cholesterol for 2 months. Histological evaluation in the liver was assessed by HE staining and Azan staining. mRNA expression in the liver was analyzed quantitative real-time PCR. IL-19KO showed significantly weight loss compared with WT mice. Liver weight was significantly less in IL-19KO. Histological analyses of WT showed moderate steatosis, mild inflammation, and no fibrosis, indicating that WT have steatohepatitis. IL-19KO showed significantly high ALT level compared with WT. Moreover, IL-19KO showed weaker steatosis and more severe inflammation. Specifically, IL-19KO showed pericellular fibrosis that is one of the features of NASH. Additionally, mRNA expression levels of TNF-alpha and IL-6 were significantly increased in IL-19KO. Our findings indicate that IL-19 has previously undocumented roles in the progress of fibrosis and the elimination of inflammation in the liver. IL-19KO may be a valuable tool to study the NAFLD/NASH mice model.

P.C1.04.15
Continual exit of human skin resident memory CD4 T cells that seed distant tissue sites

*M. M. Klicznik[1], P. A. Morawski[2], B. Hoellbacher[2], T. Duhen[2], S. Motley[2], **S. R. Varkhande**[1], M. Rosenblum[3], D. J. Campbell[2], I. K. Gratz[1];*
[1]University of Salzburg, Salzburg, Austria, [2]Benaroya Research Institute, Seattle, WA, United States, [3]University of California, San Francisco, San Francisco, CA, United States.

As a barrier organ the skin harbors immune cells that not only provide protection against a myriad of pathogens but also support tissue homeostasis and repair. A large proportion of these immune cells in skin are resident memory Tcells (TRM) with unique skin-tropic signatures that are thought to permanently reside in the tissue and not recirculate.
We have identified a novel population of circulating CD4$^+$CLA$^+$CD103$^+$cells in the blood of healthy humans. Using a multidimensional mass cytometry approach (CyTOF) we found that these cells have a unique skin-tropic phenotype reminiscent of skin TRM. Phenotypic and transcriptional similarities determined by conventional flow cytometry and RNA sequencing further highlighted the close relationship between CD4$^+$CLA$^+$CD103$^+$TRM and circulating CD4$^+$CLA$^+$CD103$^+$cells. Their unique cytokine production profile of IL-22 and IL-13, and low production of IL17a and IFN-γ indicates a function in skin homeostasis and repair. The presence of this unique population in circulation suggests recirculating TRMs, mobilized potentially to seed remote tissue sites.

To test this hypothesis, we performed xeno-graft experiments, by transferring human full thickness skin onto immunodeficient mice that carried an engineered human skin graft devoid of TRM. We found that CLA^+CD103^+TRM could be mobilized from the skin, enter circulation and seed a distant skin site, while preserving their phenotype. Further, upon transfer of human PBMC we detected CLA^+CD103^+cells that had seeded an engineered human skin graft. Thus, we propose that $CD4^+CLA^+CD103^+$cells found in the human blood represent a migrating population of skin TRM.
Funding: National Institute of Health (R01AI127726) awarded to D.J.C. and I.K.G..

P.C1.04.16
Defining the targets of autoimmune disease-associated variation in human Regulatory T cells

Y. Y. Wong;
University of Adelaide, North Adelaide, Australia.

Chromatin structure is known to have a major influence on gene expression by controlling transcription factor (TF) access to binding sites in non-coding regulatory enhancers and promoters. Studies have shown that majority of the autoimmune disease risk associated genetic variations are found in non-coding regions of the genome such as enhancers. Our lab has been working on the functional validation of cell-specific enhancers located at autoimmune disease risk loci, in order to explain how genetic variations at these loci contribute to disease. In view of this, identifying the TFs bound to enhancers and promoters is critical for understanding how these elements work and how disease-associated single nucleotide polymorphisms (SNPs) may influence this activity. Currently our ability to predict genomic binding sites from sequence alone is limited and functional TF binding sites needs to be experimentally validated. ATAC-seq, which simultaneously probe chromatin structure and importantly transcription factor binding, requires relatively few cells making it amenable to use on rare population and small clinical samples.

P.C1.04.17
Functional characterization of T lymphocytes in coeliac patients with dermatitis herpetiformis: evidence of cross reactivity between tissue and epidermal transglutaminases

M. Capone, M. Caproni, M. Rossi, A. Mazzoni, B. Rossettini, G. Montaini, G. Lami, ***L. Maggi****, A. Calabrò, L. Cosmi, F. Liotta, F. Annunziato;*
University of Florence, Florence, Italy.

INTRODUCTION: Dermatitis herpetiformis (DH) is considered the cutaneous manifestation of coeliac disease (CD), however why only few coeliac patients develop this disorder is still unknown. Epidermal transglutaminase (TG3) has been recently described as the main autoantigen of DH. Here we propose to study phenotypic features and antigen specific response of T cells towards both tissue (TG2) and epidermal transglutaminases in DH compared to CD patients. METHODS: Mononuclear cells from peripheral blood (PBMNC), skin and gut biopsies of 14 DH patients and 8 CD patients were policlonally expanded and evaluated by flow cytometry for expression of Th1, Th2 and Th17 cells associated markers. PBMNCs were also cultured in the presence of TG2 or TG3 and antigen specificity of expanded T cells has been evaluated after 15 days of culture. RESULTS: Flow cytometric evaluation showed an increased frequency of TNF-alpha producing T cells in skin of DH patients compared to CD, thus confirming the inflammatory status in the epidermal district. More interestingly, antigen specificity assays revealed that a cross reactivity occurs between TG2 and TG3 specific T cells, more in DH than in CD patients. CONCLUSIONS: Our data, even if preliminary, provide possible explanation to the mechanisms leading to DH only in some CD patients. Moreover, based on the finding of the increased production of TNF-alpha at skin level, new possible treatments should be proposed, including biological therapy, being DH very slow to respond to gluten free diet.

P.C1.04.18
Linking diet, gut immunity and microbiota in the pathogenesis of Type 1 Diabetes

I. Cosorich[1]*, L. De Giorgi*[1]*, A. Bolla*[2]*, R. Ferrarese*[1]*, E. Bosi*[2]*, R. Zuppardo*[1]*, A. Mariani*[2]*, D. Esposito*[2]*, M. Falcone*[1]*;*
[1]*San Raffaele Scientific Institute, Milan, Italy,* [2]*San Raffaele Hospital, Milan, Italy.*

Recent data indicate that gut immunity and the mechanisms that regulate effector and regulatory T cell differentiation in the intestine are instrumental to maintain immune tolerance towards self-tissues and to prevent extra-intestinal autoimmune diseases. This observation led to the hypothesis that environmental factors as diet and microbiota modifications, affect the pathogenesis of autoimmune Type 1 Diabetes (T1D).
To this aim, we analyzed gut mucosal immunity in tissue samples isolated from the small intestine of T1D patients and healthy controls (HC). A phenotypical analysis of gut mucosal immune cell subsets has been performed. We observed a statistically significant increase of Th22cells and CD1c+CX3CR1+DCs in the gut of T1D. In order to find if there is a correlative link between diet and Th subsets, we are collecting a 3-days-food record questionnaire from T1D patients. Gut microbiota of brushing material from duodenum was analyzed by 16S rRNA sequencing.
We also investigated if different type of diet can influence autoimmunity in preclinical models of T1D. In particular if an anti-inflammatory diet enriched in fibres and omega3 can reduce gut inflammation and protect NOD mice from T1D. We demonstrated a lower gut permeability in NOD mice fed with omega3 diet, compared to NOD mice fed with control diet.
We further aim at elucidating the link of gut immunity alterations and environmental factors that might have a strong impact on T1D.

P.C1.04.19
Lymph node stromal cells confer location-dependent tolerogenic properties to dendritic cells

J. Pezoldt[1]*, M. Szente-Pasztoi*[2]*, M. Zou*[1]*,* ***C. Wiechers***[1]*;*
[1]*Helmholtz Centre for Infection Research, Braunschweig, Germany,* [2]*Biomedical Center, Ludwig-Maximilians University, Munich, Germany.*

The balance between regulatory T cells (Tregs) and effector T cells is key for the maintenance of immune homeostasis. To dissect the contribution of lymph node (LN) stromal cells to T cell differentiation and peripheral *de novo* Treg induction in gut-draining mesenteric lymph nodes (mLNs), LN transplantation experiments have been utilized in a mouse model. Remarkably, the high Treg-inducing capacity of mLNs was retained after transplantation into the popliteal fossa, a non-tolerogenic, skin-draining site, although the hematopoietic compartment was completely replaced after 8 weeks, suggesting mLN stromal cells were stably imprinted with the tolerogenic properties. Additionally, the composition of incoming resident (res) dendritic cells (DCs) and their transcriptional signatures in transplanted mLN closely resemble resDCs from endogenous mLNs. Furthermore, co-cultures of resDCs re-isolated from transplanted mLN with naïve $CD4^+$ T cells from DO11.10 mice resulted in a high Treg induction, demonstrating that resDCs can get modulated by LN stromal cells after their LN entry, and that mLN stromal cells via modulation of resDCs affect local T cell differentiation and particularly *de novo* Treg induction. Finally, single-cell RNAseq data from either pLN or mLN $CD45^-CD24^-$ stromal cells revealed a to date underappreciated location-dependent heterogeneity. To discern the imprinted tolerogenic properties of stromal cells from different LNs, *in vitro* experiments of pLN vs mLN stromal cell subsets with precursor-DCs (pre-DCs) are currently underway. In conclusion, our recent data demonstrated a cross talk between LN stromal cells and resDCs, which is in turn affecting the generation and/or homeostasis of the Treg compartment.

P.C1.04.20
Fine characterization of healthy conjunctiva: main findings when comparing IELs and peripheral blood fine lymphocytes subsets

A. Corell[1]*, J. Zarzuela*[1]*, C. Martin*[2]*, A. Armentia*[1]*, R. Reinoso*[1]*, S. Rubio*[1]*, M. Cano*[1]*, A. Vallelado*[1]*, J. Herreras*[1]*;*
[1]*Universidad de Valladolid, Valladolid, Spain,* [2]*Centro de Hemoterapia y hemodonación Castilla y León, Valladolid, Spain.*

Introduction: As occurs in other mucosal tissues —for example gut, bronchi and nose—, ocular mucosa holds a conjunctiva- associated lymphoid tissue (CALT). It is well known that MALT (Mucosa Associated Lymphoid Tissue) has morphological and functional variations across tissues. Therefore, a thorough analysis of lymphoid populations might render useful information on ocular surface conditions. **Objectives:** The aim of this study is to improve the knowledge of human immune system within the conjunctiva in healthy and different ocular surface conditions. **Material and methods:** Twenty-five healthy volunteers were recruited. Peripheral blood lymphocytes were obtained by venipuncture while intraepithelial lymphocytes (IELs) from eye conjunctival mucosa were obtained by brush cytology. Major and fine subsets were characterized by flow cytometry.Memory, naïve, γδ T cells, $CD8^+$ (**Tc, NKT subtypes**), $CD4^+$(**Th0, Th1, Th2, Th17,Th1/Th17, Th22** and **Treg** subsets), B cells (**B_1** and **B_2**) and **NK cells** —regulatory and cytotoxic— subsets were analyzed in both conjunctival mucosa and peripheral blood. **Results:** Age and sex seemed to determine few differences in some lymphocyte subsets: Th1 cells might be age-influenced whereas Th22 might be sex-influenced. As expected, no strong correlations between peripheral and conjunctival lymphocytes were found. Conjunctival T cells seemed to be mainly CD8+ and TCRγδ+, while they were only a minor population in peripheral blood. Memory CD4+ T cells, NKT, B1, Tregs and regulatory NK cells had higher values in conjunctiva. **Conclusions:** Some known differences (increased TCRγδ cells) were found, whereas others are apparently new (increased B1, NKT, Tregs and regulatory NK lymphocytes) in conjunctival MALT. These suggest specialized functions (including regulatory) in the area.

P.C1.05 Maintenance and local regulation of tissue specific immunity - Part 5

P.C1.05.01
Neutrophil activation by immunoglobulin A exacerbates pathogenesis of inflammatory bowel disease

***A. Bos**, M. Bögels, R. Mebius, M. van Egmond;*
VU medical center, Amsterdam, Netherlands.

Immunoglobulin A (IgA) is the most prominent antibody in the mucosa and important for maintaining homeostasis. However, altered IgA repertoires have been found in chronic inflammatory diseases. For instance, patients with inflammatory bowel disease (IBD) have altered IgA against commensal bacteria. Therefore, this study aims to determine the contribution of IgA on IBD pathology.
We used a DSS-induced colitis model to investigate the development of colitis in human IgA x human IgA Fc receptor (hIgA$^+$/hFcαRI$^+$) mice. Compared to control mice, hIgA$^+$/hFcαRI$^+$ mice showed more severe inflammation, reduced body weight and worse survival. Additionally, massive accumulation of intra-intestinal FcαRI$^+$ neutrophils was observed. Similarly, inflamed patient biopsies revealed significant infiltration of neutrophils, together with a destroyed epithelial lining. Therefore we assessed the effect of IgA-activated neutrophils on epithelial cell line co-cultures. We observed that IgA-activated neutrophils from IBD patients form neutrophil extracellular traps (NETs) that capture epithelial cells. Importantly, we assessed IBD plasma for the presence of anti-epithelial antibodies and found specifically IgA antibodies recognizing epithelial cell lines. Nevertheless, no enhanced IgG or IgM antibodies were found against epithelial cells within these patients.Thus, we propose that anti-epithelial IgA contributes to IBD pathology by binding to the human epithelial lining, which activates neutrophils and initiates perpetual neutrophil activation, which results in massive tissue damage.

P.C1.05.02
Tissue resident memory cells T cells in the human conjunctiva and immune signatures in human ocular surface diseases.

***T. Bose**[1], L. Tong[2], G. K. Chandy[3];*
[1]Ludwigs-Maximilians-Universität, München, Germany, [2]Singapore Eye Research Institute, Singapore, Singapore, [3]Lee Kong Chian School of Medicine, Singapore, Singapore.

Non-recirculating resident memory (T_{RM}) and recirculating T cells mount vigorous immune responses to both self and foreign antigens in barrier tissues like the skin, lung and gastrointestinal tract. Using impression cytology followed by flow cytometry we identified two T_{RM} subsets and four recirculating T-subsets in the healthy human ocular surface. In dry eye disease, principal component analysis (PCA) revealed two clusters of patients with distinct T-cell signatures. Increased conjunctival central memory and naïve T cells characterized Cluster-1 patients, and increased CD8$^+$ T_{RM}s and CD4$^+$ recirculating memory T cells characterized Cluster-2 patients. Interestingly, these T-cell signatures are associated with different clinical features: the first signature correlated with increased ocular redness, and the second with reduced tear break up times. These findings open the door to immune-based characterization of ocular surface disease and T-subset specific immunotherapies to suppress T-subsets involved in disease. They may also help with patient stratification during clinical trials of immunomodulators.

P.C1.05.03
The local microenvironment drives the identity of tissue-resident lymphocytes

***S. Christo**[1], M. Evrard[1], D. Newman[1], S. L. Park[1], J. E. Prier[1], F. R. Carbone[1], F. Ginhoux[2], A. Kallies[1], L. K. Mackay[1];*
*[1]The Peter Doherty Institute, Melbourne, Australia, [2]Signapore Immunology Network (SIgN) Agency for Science, Technology and Research (A*STAR), Singapore, Singapore.*

Tissue-resident memory T (T_{RM}) cells are a population of non-recirculating lymphocytes that permanently reside in non-lymphoid organs. Together with other tissue-resident lymphocytes, these cells are critical for controlling infection and cancer, and are implicated in promoting tissue repair and autoimmunity. Although T_{RM} cells possess a common molecular signature distinguishing them from their circulating counterparts, the transcriptional identity of these cells differs vastly between organs. We found that organ-specific gene signatures are conserved between different subsets of resident lymphocytes within a given tissue, partly reflective of local cytokine imprinting. We show that whilst T_{RM} cells in different tissues exhibit differential cytokine and molecular requirements for their development, locally-derived factors cooperate to globally suppress tissue-egress genes in resident cells across all organs. Collectively, our data demonstrate the adaptation of T_{RM} cells to specific tissue microenvironments. Exploiting such commonalities and differences in T_{RM} cell regulation will inform new strategies designed to target these cells in a site-specific manner.

P.C1.05.04
Resident T cells trigger disease-associated tissue responses that stratify clinical outcome in human psoriasis

*I. Gallais-Sérézal, S. Cheuk, **L. Eidsmo**;*
Department of Medicine Solna, Stockholm, Sweden.

Resident T cells provide barrier immunity in murine models of viral infections. In contrast, alteration in functionally distinct subsets of resident T cells is implicated in human focal skin diseases. We recently reported that CD49a marks CD8+ T cells poised to cytotoxicity and IFN-gamma production, while IL-17 producing T cells form localised disease memories in resolved psoriasis. However, if and how these cells cause human pathology is not known. Here, we investigated the consequences of T cell activation on tissue response patterns. T cell activation induced type-1 interferon tissue responses in explanted skin tissue and psoriasisform, IL-17-related responses were selectively induced in psoriasis-derived skin. Our data indicates that local T cell activation induce clinically relevant tissue responses and stratification of these responses in resolved psoriasis were correlated to clinical outcome. Finally, our data indicates that microbial interplay with genetically predisposed keratinocytes may shape the local pool of resident T cells.

P.C1.05.06
***Helicobacter hepaticus* as disease driver in a novel CD40-mediated spontaneous colitis-model**

***V. Friedrich**[1,2], C. Barthels[1], A. Ogrinc[1], D. Garzetti[3], B. Stecher[3], I. Forné[4], A. Imhof[4], T. Brocker[1];*
[1]Institute for Immunology, Biomedical Center, Ludwig-Maximilian-University, Munich, Germany, [2]Graduate School of Quantitative Biosciences (QBM), Ludwig-Maximilian-University, Munich, Germany, [3]Max von Pettenkofer-Institute, Ludwig-Maximilian-University, Munich, Germany, [4]Protein Analysis Unit, Biomedical Center, Ludwig-Maximilian-University, Munich, Germany.

The mammalian gastrointestinal tract is shaped by microbiota. To maintain mucosal homeostasis, a balance between appropriate immune responses to invading pathogens and tolerance to food and commensal-derived antigens is essential. Disturbed balances can result in severe inflammatory disorders like Inflammatory Bowel Disease. Dendritic cells (DCs) play a key role in this regulation as they can induce both, immunity and tolerance. To investigate the role of the CD40L-CD40 axis in tolerance vs. immunity and the role of DCs therein, we generated a murine model with constitutive CD40-signaling in DC. CD40-signaling leads to migration of CD103$^+$ DCs from the colonic lamina propria to draining lymph nodes, followed by DC-apoptosis. This loss of CD103$^+$ DCs caused lack of RORγt$^+$Helios$^-$ induced regulatory T cells and an increase of Th1/Th17 effector cells in the colon, resulting in breakdown of mucosal tolerance and severe colitis.
We used sera from these mice to isolate fecal antigens recognized by mice with colitis, but not control mice and studied changes of the microbiota during disease development. We detected *Helicobacter hepaticus (H.h.)*-specific antibodies in transgenic mice and could protect them from disease onset by rendering them *H.h.*-free. Upon *H.h.*-reinfection of transgenic mice, rapid disease onset was observed. Our data suggest that *H.h.* is the disease driver in a CD40-mediated spontaneous colitis-model, allowing us to study T cell specificities and differentiation plasticity during inflammation more in detail.

P.C1.05.07
Canonical and non-canonical functions of tyrosine kinase 2 during liver inflammation

***D. Gogova**[1], C. Lassnig[1,2], S. Knapp[3], A. Puga[1], M. Müller[1], B. Strobl[1];*
[1]Institute of Animal Breeding and Genetics, Vienna, Austria, [2]Biomodels Austria, University of Veterinary Medicine Vienna, Vienna, Austria, [3]CeMM (Research Center for Molecular Medicine of the Austrian Academy of Sciences) and Laboratory of Infection Biology, Department of Medicine I, Medical University, Vienna, Austria.

Tyrosine kinase 2 (TYK2) belongs to the Janus kinase family of receptor-associated tyrosine kinases and is an integral part of signalling cascades utilized by many cytokines with important immune regulatory activities. Mice deficient for TYK2 (*Tyk2*$^{-/-}$) or expressing enzymatically inactive TYK2 (*Tyk2*^{K923E}) allow us to distinguish between kinase-dependent and independent functions in immune responses.
We found that TYK2 and its kinase activity are required to protect from *Escherichia coli*-induced liver injury, which correlated with diminished systemic and hepatic levels of interleukin-22 (IL-22). IL-22 signals through TYK2, is produced by innate and adaptive immune cells upon stimulus with various cytokines and growth factors and has been reported to have hepatoprotective effects. Next, we employed the concanavalin A (ConA)-induced acute hepatitis model to further study the role of TYK2 in IL-22 production and signalling, as previous studies showed increased disease severity in *Il22*$^{-/-}$ mice. We show that *Tyk2*$^{-/-}$ mice have significantly increased liver damage parameters and decreased systemic and hepatic levels of IL-22 compared to wild-type mice. Surprisingly, despite diminished levels of IL-22, *Tyk2*^{K923E} mice showed liver damage comparable to wild-type mice. Analysis of disease-driving cytokines revealed that hepatic *Ifng* mRNA expression was almost completely abolished in *Tyk2*^{K923E} and *Tyk2*$^{-/-}$ mice, whereas *Tnfa* expression was specifically reduced in *Tyk2*^{K923E} animals.
Taken together, we show that TYK2 protects from infection- and inflammation-induced hepatitis and that TYK2^{K923E} is capable to prevent inflammatory TNFα production and ameliorate liver injury.
Supported by FWF grants P25642-B22, SFB-F6101 and SFB-F6106.

P.C1.05.08

A single nucleotide polymorphism in the promoter region of the inhibitory immune receptor SIRL-1 controls its surface expression on mononuclear phagocytes

D. Gollnast[1], *T. van Capel*[2], *B. Giovannone*[1], *M. van der Vlist*[1], *D. Hijnen*[3], *E. de Jong*[2], *L. Meyaard*[1];
[1]*Laboratory of Translational Immunology (UMCU), Utrecht, Netherlands,* [2]*Academic Medical Center, Amsterdam, Netherlands,* [3]*Dermatology and Allergology (UMCU), Utrecht, Netherlands.*

Signal inhibitory receptor on leukocytes-1 (SIRL-1) is expressed highly on human blood granulocytes and monocytes and low on circulating myeloid dendritic cells (mDCs) and basophils. SIRL-1 ligation inhibits innate effector mechanisms such as production of FcR-induced reactive oxygen species in monocytes and neutrophils and NETosis in neutrophils. We show that the single nucleotide polymorphism (SNP) rs612529T/C, located in the SIRL-1 promoter region, abrogates SIRL-1 expression in monocytes and dendritic cells, whereas expression levels in neutrophils and eosinophils remain unaffected.
Using targeted association analysis, rs612529T/C could be associated to the skin inflammatory disease atopic dermatitis. By FACS analysis we found that in healthy skin, SIRL-1 is restricted to very low expression on subpopulations of skin-resident DCs. By RT-PCR analysis of FACS-sorted skin-resident immune cells we identified SIRL-1 mRNA exclusively in dermal mDCs with elevated levels in $CD14^+$ DC-like macrophages. In individuals that carry the rs612529C allele, SIRL-1 transcripts were undetectable in these dermal cell types, indicative of similar transcriptional regulation of SIRL-1 in skin-resident DCs as in blood-resident monocytes and mDCs.
In contrast to skin, high expression of SIRL-1 was detected on 30-50% of tissue-resident immune cells in healthy lung tissue samples derived from tumor-removal surgery. SIRL-1 was expressed on interstitial macrophages, monocytes, and mDC subsets.
These results suggest a role of SIRL-1 in immune regulation of mononuclear cells, including monocytes, DCs and macrophages. Immune inhibitory functions of SIRL-1 may be important to circumvent the manifestation of inflammatory diseases in human barrier tissues and may be of particular importance in lung.

P.C1.05.09

Massive and organized B-cell infiltrates in the aorta of LV-GCA patients

J. C. Graver*, M. Sandovici, E. A. Haacke, A. M. Boots, E. Brouwer;*
University of Groningen, University Medical Center Groningen, Groningen, Netherlands.

Giant cell arteritis (GCA) is the most common type of systemic vasculitis and can be classified into cranial(C)-GCA and large-vessel (LV)-GCA. GCA is postulated to be T-cell-mediated and in temporal artery infiltrates, T-cells clearly outnumber B-cells. However, our report on a disturbed homeostasis of B-cells in newly diagnosed GCA patients shows evidence for a putative role of B-cells. So far, the role of B-cells is underexplored and the presence of B-cells in the vessel wall of LV-GCA patients is unknown. Therefore, this study assessed the presence of B-cells in the aorta of LV-GCA patients.
Aorta tissue samples of 9 histologically-proven LV-GCA patients who underwent surgery due to an aortic aneurysm were studied by immunohistochemistry. Staining was performed with antibodies against CD20 (B-cells), CD3 (T-cells), CD21 (follicular dendritic cells (FDC)), bcl6 (germinal centers), and CD138 (plasma cells). None of the patients received immunosuppressive treatment at the time of surgery. Aorta tissue from age- and sex-matched atherosclerosis patients with an aneurysm were included as controls.
Aorta tissues of LV-GCA patients showed massive infiltration of B-cells, mainly in the adventitia. In contrast to the temporal artery, B-cells outnumbered T-cells in the aorta. B-cells organized into artery tertiary lymphoid organs; there was co-localization of B- and T-cells, FDCs, germinal centers and plasma cell niches.
Aorta tissues from patients with histologically-proven LV-GCA showed massive and organized B-cell infiltrates in the adventitia. The mere presence of B-cells at the site of inflammation prompts further investigation into the role of B-cells in GCA.

P.C1.05.10

Tryptophan metabolism in inflammatory bowel disease - Distribution and regulation of enzymes, metabolites and target structures in a multidimensional model

M. Huhn[1], *M. Herrero San Juan*[1], *R. Boll*[2], *J. Pfeilschifter*[1], *P. Weller*[2], *H. Radeke*[1];
[1]*pharmazentrum frankfurt, Frankfurt am Main, Germany,* [2]*Institute for Instrumental Analysis and Bioanalytic, Mannheim, Germany.*

The immune pathogenesis of inflammatory bowel diseases leads to a perpetuating mucosal inflammation based on an imbalance of pro- and anti-inflammatory cytokines. While tryptophan (TRP) and kynurenine have been examined in detail, neither downstream enzymes nor other cells involved in chronic inflammation have been studied in detail. Therefore, we sought to complete the knowledge of downstream enzymes in all cell types involved in chronic inflammation. We performed a multidimensional metabolome TRP analysis in primary colon cell lines, cancer cell lines and immune cells of healthy donors by LC-MS/MS and qRT-PCR. Cells of the innate immune system, especially monocytes, dendritic cells and macrophages, were proven to be the main producers of TRP metabolites. In addition, mRNA of indoleamine 2,3-dioxygenase 1 (IDO1) was expressed in cytotoxic CD8+ T cells and B cells. Colon cells revealed no constitutive expression of IDO1 and IDO2 mRNA. Tryptophan 2,3-dioxygenase (TDO) and Kynurenine 3-monooxygenase (KMO) mRNA were detected in cancer cell lines CaCo-2 and DLD-1, but not in primary colon cell line CCD841 CoN. Furthermore, mRNA expression of G protein-coupled receptor (GPR35) was shown to be increased in CaCo-2 and DLD-1. However, only low expression levels of GPR35 mRNA were detected in CCD841 CoN. Our findings reveal an altered TRP metabolism, with an increased activity caused by inflammatory conditions. Taken together, we identified TDO, KMO and GPR35 as potential biomarkers for colorectal cancer. These results point to a major role in the development and progression of chronic diseases like cancer. Funded by the Arbeitsgemeinschaft-industrieller-Forschungsvereinigungen Germany.

P.C1.05.11

A trauma and victimization history associate with Immune barrier dysregulation in women

A. S. Kohlmeier[1], *L. B. Haddad*[2], *K. A. Brookmeyer*[1], *J. M. Baker*[3], *K. Chi*[1], *C. Y. Chen*[1], *E. N. Kersh*[1], *J. A. Johnson*[1], *M. M. Herbst-Kralovetz*[4], *M. Hogben*[1], *I. Ofotokun*[2], *J. E. Kohlmeier*[2];
[1]*CDC, Atlanta, United States,* [2]*Emory University, Atlanta, United States,* [3]*The University of Arizona, Pheonix, United States,* [4]*The University of Arizona, Phoenix, United States.*

Background: A growing body of literature suggests women who have experienced psychological traumas such as violence victimization, are more vulnerable to sexually transmitted infections (STIs). Though this susceptibility has been associated with sexual behaviors, a biological impact on STI risk through long-lasting immune changes at sites of STI exposure has not been investigated. We examined immune barrier composition and lifetime trauma and victimization history (LTVH) by characterizing critical cellular immune mediators at the apical lumen of the lower FRT. Results: Samples from high (n=20) compared to low (n=22) LTVH scoring participants presented alterations in barrier homeostasis and cell composition at the FRT lumen. Specifically, we found increased detection of MHC class II+ antigen presenting cells, expressing increased frequencies of CCR5 and CD103. Corresponding to this observation local T cell populations, expressed reduced frequencies of the tissue retention marker CD69 and increased frequencies of lymphoid trafficking marker CCR7. Additionally, genes involved in maintaining tight junctions and epithelial barrier integrity ALOX12, OCLN, LCE3D were decreased in FRT epithelial cells. Conclusions: Repeated exposures to trauma and violence victimization was associated with alterations in barrier homeostasis and cell composition. While low scoring participant samples expressed characteristics of a healthy immune-restricted barrier, samples from high-scoring participants exhibited immune perturbations that evidence compromised immune defenses to initial pathogen encounter. These data indicate that alterations in immune mediators at the FRT may directly contribute to an increased risk of STIs among women who endure greater trauma and violence victimization.

P.C1.05.12

Understanding the crosstalk between immune and epithelial cells in the development of the "TTP-deficiency" syndrome

C. La[1], *B. De Toeuf*[2], *A. Assabban*[1], *H. Shehade*[1], *P. J. Blackshear*[3], *L. Van Maele*[1], *C. Gueydan*[2], *G. Oldenhove*[2], *S. Goriely*[1];
[1]*Institute for Medical Immunology, Gosselies, Belgium,* [2]*Laboratory of Molecular Biology of the Gene, Institut de Biologie et de Médecine Moléculaire, Gosselies, Belgium,* [3]*Duke University Medical Center, Durham, United States.*

Introduction. Interactions between epithelial barriers and environmental stimuli are essential for a functional immunity, to maintain homeostasis and avoid tissue damage. Tristetraprolin (TTP, encoded by *Zfp36*) is an RNA-binding protein, regulating inflammatory genes (eg. *Tnf*, *Il23a*, *Cxcl2)*. TTP deficient mice develop a spontaneous inflammatory syndrome, which had been largely attributed to dysregulated function of myeloid cells. However, mice harboring conditional TTP deletion in myeloid (LysM-*Zfp36*$^{fl/fl}$) or dendritic cells (CD11cCre-*Zfp36*$^{fl/fl}$) do not develop any spontaneous pathology. In sharp contrast, TTP deletion in keratinocytes (K14Cre-*Zfp36*$^{fl/fl}$) is sufficient to trigger the "TTP-deficiency" syndrome. Our goal is to further define the role of environmental factors in this pathology and the role of TTP in gut homeostasis. **Results.** 1) This syndrome was more severe under conventional housing compared to SPF facility. 2) Unexpectedly, TTPKO mice derived on MyD88- or Caspase1-deficient backgrounds, involved in key signaling pathways towards microbial recognition, developed more severe inflammation, pointing to a potential role of dysbiosis and inadequate response at epithelial barriers. 3) We observed an overgrowth of the *Enterobacteriaceae* family in the gut of TTPKO mice. 4) There was no overt intestinal inflammation histologically but mRNA expression of cytokines and inflammatory markers was increased in the ileum. We thus hypothesize that absence of TTP might also activate regulatory mechanisms controlling this subclinical inflammation. Indeed we observed higher levels of IL-22 and increased Tregs in the lamina propria. **Perspectives.** To further study the role of TTP in epithelial (using VillinCre-*Zfp36*$^{fl/fl}$ mice) and immune cells and the involvement of the gut microbiota.

P.C1.05.13

ASC in CD4$^+$ T cells intrinsically limits their proliferation capacity and is required to maintain intestinal homeostasis

*H. Javanmard Khameneh[1], **A. Mortellaro**[1,2];*
*[1]Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Singapore, Singapore, [2]San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), IRCCS San Raffaele Scientific Institute, Milan, Italy.*

The apoptosis-associated speck-like protein containing a CARD (ASC or Pycard) plays a pivotal role in the assembly and activation of the inflammasome complex. Inflammasome activation is essential for caspase-1 activation in response to diverse danger and pathogen-associated signals, which leads to proteolytic cleavage and release of the pro-inflammatory cytokines IL-1beta and IL-18. ASC has been shown to play important roles in the context of inflammation, cell death, and tumorigenesis. While ASC has been broadly implicated in inflammasome activation in myeloid cells, such as macrophages and neutrophils, little is known about its contribution in lymphocyte biology. Here, we found that Asc is expressed in naïve T cells and its loss resulted in increased proliferation *in vitro* and *in vivo*, indicating that ASC intrinsically fine-tunes proliferative capacity of CD4$^+$ T cells. Using a mouse model of induced chronic colitis, we found that ASC expression in CD4$^+$ T cells intrinsically suppresses their colitogenic capacity, facilitating the maintenance of gut homeostasis by the mucosal adaptive immune system. Further analysis of Asc$^{-/-}$ CD4$^+$ T cells revealed a stronger TCR signaling and an altered metabolic profile in these cells compared to wild-type CD4$^+$ T cells. In conclusion, ASC in CD4$^+$ T cells has crucial non-inflammasome functions to modulate T-cell biology and maintenance of mucosal immune homeostasis in the gut.

P.C1.05.14

The co-inhibitory molecule PD-L1 contributes to regulatory T cell-mediated protection in murine crescentic glomerulonephritis

***K. Neumann**[1], A. Ostmann[1], P. C. Breda[1], A. Ochel[1], F. Tacke[2], H. Paust[1], U. Panzer[1], G. Tiegs[1];*
[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2]RWTH-University Hospital Aachen, Aachen, Germany.

Introduction: Crescentic glomerulonephritis is a severe glomerular disease mediated by inappropriately regulated cellular and humoral immune responses subsequently leading to development of end-stage renal failure. Previously, we demonstrated a crucial role for regulatory T cells (Treg) in controlling the inflammatory Th1 immune response during nephrotoxic nephritis (NTN), the murine model of crescentic glomerulonephritis. Here, we aim at investigating the role of the co-inhibitory molecule PD-L1 in Treg-mediated protection from NTN.
Methods: NTN was induced by i.p. injection of nephrotoxic serum. Analysis was done eight days later. Kidney damage was analyzed by quantification of crescent formation and determination of albumin-to-creatinine ratio. Tregs from nephritic PD-L1$^{-/-}$ and WT mice were transferred into Rag1$^{-/-}$ mice one day before NTN induction. Cytokine expression was analyzed by quantitative RT-PCR and flow cytometry.
Results: We demonstrated that Foxp3$^+$ Tregs expressing PD-L1 infiltrate the kidney during NTN. In nephritic *PD-L1$^{-/-}$* mice, the frequency of renal Tregs was increased compared to nephritic WT mice. However, *PD-L1$^{-/-}$* mice developed more severe NTN associated with a strongly elevated renal Th1 immune response. The same findings were shown after blockage of PD-L1 in WT mice. Moreover, neutralization of IFNγ in PD-L1$^{-/-}$ mice ameliorated NTN. Interestingly, lack of PD-L1 profoundly altered the gene expression profile of Tregs in homeostasis and kidney inflammation. Functionally, Tregs from nephritic PD-L1$^{-/-}$ mice had impaired suppressive capacity *in vitro* and did not protect from NTN *in vivo*.
Conclusion: PD-L1 displays a protective role in NTN, which is related to Treg-mediated suppression of the Th1 immune response.

P.C1.05.15

MicroRNAs as a regulator of development and progression in inflammatory bowel diseases among the Polish population.

***A. Surowiecka-Pastewka**[1,2], E. Zakościelna[1], M. Zagozda[1], M. Durlik[1,2];*
[1]Mossakowski Medical Research Centre of the Polish Academy of Sciences, Warsaw, Poland, [2]Department of Gastroenterological Surgery and Transplantation, Warsaw, Poland.

Introduction: Crohn's disease (CD) and ulcerative colitis (UC) are inflammatory bowel diseases(IBD) characterized by chronic inflammation of the gastrointestinal tract. The immunopathogenesis of the diseases is still not completely understood, however it has been proven that miRNAs have a key regulatory role in the development of IBD. Therefore, the aim of this study was to compare the expression of selected miRNAs with clinical data within patients with IBD. **Materials and Methods:** This study consisted of 60 IBD patients, as well as 50 healthy controls. The expression patterns of the circulating miRNA in serum were quantitatively assayed using reverse transcription and real-time RT-PCR.
The results were analyzed using Statistica software. **Results:** We detected significantly up-regulated expression of miR-21 in a serum isolated from IBD patients. Genotype-phenotype correlation analysis revealed thatmiRNA expression was associated with severe form of the disease.
Moreover, miRNA expressionwas associated with the development of perianal fistulas compared to control. **Conclusions:** Changes in the levels of miR-21 may suggest its involvement in the development and progression IBD. We demonstrate that circulating miRNAs correlate with disease activity and may be considered as potential tool for the further biomarker research in IBD. However, a thorough confirmation of these mechanism require further investigation.

P.C1.05.16

Gammapathy, immunodeficiency and autoimmunity: when the immune system turns against us

***P. E. Walo Delgado**, P. Lapuente-Suanzes, I. Nieto-Gañan, A. Carrasco-Sayalero;*
Servicio de Inmunología. Hospital Universitario Ramón y Cajal, Madrid, Spain.

Introduction. The immune system helps to keep homeostasis. It is capable of recognizing, controlling and eliminating pathogens as well as neoplastic cells. When a dysfunction of any of its components exists, immune tolerance, as well as other pathways of the immune system, may result affected in a non-specific manner, predisposing to the appearance of autoimmune disease, immunodeficiencies, allergies and lymphoproliferative disorders.
Description: We present the case of a 54-year-old man with no relevant medical history who attended to the emergency department with temporal-spatial disorientation and seizures during the course of an influenza A H1N1 infection. Anti-amphiphysin antibodies were identified, without evidence of neoplasm after thorough testing was performed. Also, biclonal IgG kappa and lambda gammapathy was detected by isoelectric focusing, associated with pan-hypogammaglobulinemia of polyclonal IgG, IgA and IgM, with decreased circulating memory B-cells measured by flow cytometry. One year later, he suffered an ischemic stroke and status epilepticus that was ruled to be of possible autoimmune etiology; however, no anti-neuronal antibodies were identified this time. In the following years, he has developed several episodes of encephalitis and recurrences of disseminated herpes zoster.
Conclusion. Despite not being able to find a clear causal correlation between the episodes of autoimmune encephalitis and the several other immunological alterations shown by the patient, it could be hypothesized that the presence of autoimmunity, as well as hypogammaglobulinemia in this case – causing increased susceptibility to infections- is found in the context of biclonal gammopathy, thus constituting a well-known, classical association between autoimmunity and immunodeficiency.

P.C1.05.17

Characterizing intraepithelial lymphocytes in human bile ducts

***C. L. Zimmer**[1], E. Von Seth[2,3], O. Strauss[1], L. Berglin[1], U. Arnelo[2], M. Hansson[2], A. Bergquist[2,3], N. K. Björkström[1];*
[1]Center for Infectious Medicine, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden, [2]Department of Hepatology, Karolinska University Hospital, Stockholm, Sweden, [3]Unit of Gastroenterology and Rheumatology, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.

The bile duct is a mucosal barrier tissue connecting the liver with the small intestine. Intraepithelial lymphocytes (IELs) typically reside in epithelial layers of barrier tissues and are thought to play a role in the immunopathogenesis of diseases in bile ducts, such as primary sclerosing cholangitis (PSC), a chronic inflammatory disease with unknown etiology. However, the composition and function of immune cells localizing to bile ducts remain unexplored. Using endoscopy, we obtained brush-samples from the bile duct mucosal surface and carried out an extensive flow cytometric analysis of the biliary immune system. Our results revealed major differences in immune cell composition in bile ducts as compared to peripheral blood. This included the presence of a sizeable population of CD69$^+$CD103$^+$ IELs that was varified using immunofluorescencent microscopy. These IELs had a TCRαβ$^+$CD8αβ$^+$ effector memory T cell phenotype, presented with a gut and liver homing chemokine receptor profile, and were highly functional. Taken together, the characterization of the biliary immune system increases our understanding of immune responses at this previously unexplored site and sheds new light on the immunopathogenesis of PSC.

P.C1.05.18

Dynamic transcriptional and epigenetic response of CD8 + intraepithelial lymphocytes (IELs) to proinflammatory cytokines

M. M. Zorro Manrique[1], *R. Aguirre-Gamboa*[1], *T. Mayassi*[2], *C. Ciszewski*[2], *C. Wijmenga*[1], *S. Withoff*[1], *B. Jabri*[2], *Y. Li*[1], *I. Jonkers*[1];
[1]*UMCG, Groningen, Netherlands,* [2]*University of Chicago, Chicago, United States.*

Cytokine deregulation contributes to the development of autoimmune diseases (AIDs) by eliciting the activation of immune cells, including cytotoxic CD8⁺ T cells. These cells are located in mucosal and intestinal tissues, where they detect and destroy pathogens, but are also known to damage the intestinal barrier under disease conditions. Limited recovery from intestinal biopsies has hampered study of CD8⁺ Intra Epithelial Lymphocytes (IELs). To elucidate the molecular pathways involved in activation of these cells by AID-related cytokines, we investigated the dynamic transcriptomic and epigenetic changes and cytokine production of CD8⁺ IELs derived from the intestine of patients in response to IL-15, IL-21 or IFNβ at different time points (0h, 30min, 3h, 4h, and 24h).
Our results show unique gene expression profiles for each cytokine. IL-21 promoted moderate gene expression changes (838 differentially expressed genes (DEGs) after 3h), most related to immune pathways. IL-15 and IFNβ provoked strong activation of IELs. IFNβ induced a robust interferon immune response mediated by STAT1, followed by a drastic increase in gene expression of cell cycle genes. Conversely, IL-15 induced immune- and RNA-processing genes, likely mediated by AP-1 and EGR1, which were mostly restored to resting state after 24 h. Although the secretion of cytokines did not follow the patterns of expression of their encoding genes, the transcriptional profiles were mirrored by changes in H3K27Ac profiles at genes and enhancers that regulate DEGs.
In conclusion, these cytotoxic IELs show great plasticity in both epigenetic and transcriptomic profiles in response to the inflammatory milieu

P.C1.05.19

Mature naive B cells accumulate in intervillous space of term placenta and positively associate with specific chemokines

A. Lundell[1], *M. Solders*[2], *L. Gorchs*[2], *S. Gidlöf*[3], *E. Tidblad*[3], *H. Kaipe*[2];
[1]*Department of Rheumatology and Inflammation Research, Sahlgrenska Academy, Gothenburg, Sweden,* [2]*Dept of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden,* [3]*Dept of women's and children's health, Karolinska Institutet, Stockholm, Sweden.*

Introduction: Total B cell numbers in the circulation decrease during late pregnancy compared to post-partum and to non-pregnant controls, but the underlying mechanisms for this is unknown. The aim of this study was to examine if the proportions of total B cells and B cell subsets at different maturational stages differ in peripheral blood (PB) compared to placental intervillous blood (IVB) at delivery. **Methods:** From 23 paired samples of PB and IVB following uncomplicated full term pregnancies, total B cells as well as transitional, mature/naïve and memory B cells were identified by flow cytometry. Chemokine levels in blood were analyzed using a luminex assay. **Results:** We found that the proportions of total B cells, as well as the fraction of mature/naïve B cells, were significantly higher in IVB relative to PB. In contrast, the proportions of immature transitional B cells and memory B cells were higher in PB compared to IVB. Multivariate factor analysis demonstrated that a specific profile of chemokines in IVB, including CCL20, positively associated with higher proportions of mature/naïve B cells in the intervillous space. Although all B cells expressed CCR6, the corresponding receptor for CCL20, mature/naïve B cells expressed the highest levels of this receptor. Migration assays further showed that B cells migrate towards placental explant-derived supernatants. **Conclusion:** these results suggest that B cells, and mature/naïve B cells in particular, home to the placenta in response to certain chemokines produced by this tissue during late pregnancy.

P.C1.05.20

Effects of terminating treatment with ADSC-derived exosomes on obesity

Q. WANG, *H. Zhao;*
School of Basic Medical Sciences, Shandong University, Jinan, China.

We have recently reported that exosomes from adipose-derived stem cells (ADSCs) attenuate adipose inflammation, insulin resistance or even obesity development in mice fed on high-fat diet. ADSC-derived exosomes drive the polarization of macrophages into anti-inflammatory M2 phenotypes through transactivation of arginase 1, which further mediate beiging of white adipose tissue. To optimize the protocol for exosome treatment, the administration of ADSC-derived exosomes was ended in high-fat diet-fed mice that received 6-8 weeks of exosome injection, the adipose tissue inflammation and insulin resistance were evaluated. After 6-8 weeks of exosome treatment, the mice showed significant improvement on glucose tolerance; while 3 weeks after terminating treatment, ADSC-derived exosomes showed no more protection against insulin resistance in obese mice. Four weeks after terminating treatment with exosomes, the mRNA level of arginase 1 in stromal vascular fraction from epididymal fat pad showed no significant elevation despite an increasing trend, while the mRNA level of TNF-α was still significantly decreased; additionally, no obvious decreases in fat mass were observed. These findings suggest that termination of treatment partially reduces the beneficial effects of ADSC-derived exosomes on obesity-associated adipose tissue inflammation and metabolic disorders, which need to be considered during the exosome treatment for obesity. This study is supported by National Natural Science Foundation of China 81471065, 81770838 and Shandong Major Research Program 2016GSF201005.

P.C1.05.21

Tipping the scale - how a shift in the presentation of self-antigen can prime for autoimmunity

J. Petersen[1], *J. D. OOi*[2], *H. Reid*[1], *J. Rossjohn*[1], *R. Kitching*[3];
[1]*Monash University, Clayton, Australia,* [2]*Centre for Inflammatory Diseases, Clayton, Australia,* [3]*Department of Nephrology, Clayton, Australia.*

The T cell repertoire of each individual is shaped to finely balance the recognition of foreign antigen against potential autoimmunity. This balance is determined in the thymus, where the interactions between HLA presented self-antigens and maturing T cells drive T cell selection and further determine their differentiation into effector and regulatory T cells. Goodpasture disease is strongly associated with HLA-DR15, whereas individuals carrying HLA-DR1 are dominantly protected. Our recent data provides insight into how specific HLA class II alleles impact on the functional bifurcation of the T cell repertoire towards a single a self antigen, thus lending protection or predisposing for autoimmune disease (Ooi Petersen et al., Nature 2017).

P.C1.06 Maintenance and local regulation of tissue specific immunity - Part 6

P.C1.06.01

Characterizing the role of lymphatics in contact hypersensitivity

P. Aradi, *Z. Horváth, É. Kemecsei, Z. Jakus;*
Department of Physiology, Semmelweis University School of Medicine, Budapest, Hungary.

Contact hypersensitivity (CHS) reaction, the mouse model of human allergic contact dermatitis, can be induced by repeated exposure to contact allergens. The lymphatic system is a critical player for the regulation of the immune responses in infectious diseases in the skin but the possible role of lymphatics in the development of allergic contact dermatitis remains unclear.
In our studies *Flt4*$^{kd/+}$ mice carrying a germline point mutant kinase dead Vegfr3 allele were used. CHS was initiated by the exposure of the skin to TNCB (2,4,6-trinitrochlorobenzene) followed by a second treatment. The disease progression was monitored by the measurement of ear thickness. The ears were collected for paraffin-based histology followed by H&E staining and immunostaining against lymphatic and immune cell markers.
We found the complete lack of lymphatics in the skin including the ear of *Flt4*$^{kd/+}$ mice, while the lymphatic structures were present in the lung and small intestine. We characterized the development of CHS in *Flt4*$^{+/+}$ and *Flt4*$^{kd/+}$ mice and our experiments have revealed reduced inflammation in the ear of the *Flt4*$^{kd/+}$ mice. CHS development induced dynamic changes in lymphatic morphology and resulted in unexpected lymphatic growth in *Flt4*$^{kd/+}$ ears.
Our results revealed that dynamic changes of lymphatic morphology occur in CHS, and the inflammation is reduced in the Flt4$^{kd/+}$ mice lacking lymphatics in the ears. They also suggest that distinct mechanisms regulate the developmental and inflammatory lymphangiogenic program. Our findings define novel aspect of the interactions between the immune and lymphatic in allergic diseases.

P.C1.06.02

Relative efficiencies of peptidylarginine deiminase (PAD) 2 and 4 in generating target sites in fibrinogen, alpha-enolase and histone H3 for anti-citrullinated protein antibodies

D. Damgaard[1], *M. Bawadekar*[2], *L. Senolt*[3], *A. Stensballe*[4], *M. A. Shelef*[2,5], *C. H. Nielsen*[1];
[1]*Institute for Inflammation Research, Center for Rheumatology and Spine Diseases, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark,* [2]*Department of Medicine, University of Wisconsin, Madison, United States,* [3]*Institute of Rheumatology and Department of Rheumatology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic,* [4]*Department of Health Science and Technology, Aalborg University, Aalborg, Denmark,* [5]*William S. Middleton Memorial Veterans Hospital, Madison, United States.*

Introduction: Peptidylarginine deiminase 2 (PAD2) and PAD4 are expressed in the synovium of rheumatoid arthritis (RA) patients and catalyze citrullination of arginine residues in proteins targeted by anti-citrullinated protein antibodies (ACPAs). Little is known about the relative importance of PAD2 and PAD4 in generating citrullinated self-antigens. Here we investigate the ability of PAD2 and PAD4 to generate citrullinated targets of ACPAs in four human proteins.

Materials and Methods: Synovial fluid (SF) and plasma were collected from 42 RA patients. Human fibrinogen, human alpha-enolase (ENO1), human histone H3, and human serum albumin (HSA) were citrullinated *in vitro* by PAD2 and PAD4. The total degree of citrullination was determined using the anti-modified citrulline approach. Antibody binding to native and citrullinated proteins was measured by ELISA.
Results: ACPAs within pooled SF from multiple RA patients reacted equally well with PAD2- and PAD4-citrullinated fibrinogen, at any PAD and SF concentration used. Accordingly, ACPAs from most individual patient SF and plasma samples bound equally well to PAD2- and PAD4-citrullinated fibrinogen and ENO1. Native ENO1 was also targeted substantially by autoantibodies. When histone H3 was used as target, PAD4 was generally superior in generating epitopes recognized by ACPAs. Despite adequate citrullination, no binding to citrullinated HSA was observed.
Conclusion: In most patients, PAD2 and PAD4 are equally efficient in generating citrullinated target sites in fibrinogen and ENO1. The binding of autoantibodies to histone H3 was generally higher after citrullination with PAD4 than with PAD2. Citrullinated HSA is not a target for ACPAs.

P.C1.06.03
Autoantibodies against the basal cell layer recognize keratin 14 and keratin 5 in patients withhepatitis delta virus

J. Delgado de la Poza, *C. García Miralles;*
Parc Taulí Hospital Universitari. Institut d'Investigació i Innovació Parc Taulí I3PT. Universitat Autònoma de Barcelona, Sabadell, Spain.

Introduction: Hepatitis delta is the most severe viral hepatitis leading to hepatic decompensation and hepatocarcinoma rapidly. Zauli D et al. in 1984 described antibodies to the basal cell layer (BCL) in patients with hepatitis delta virus (HDV). To date, anti-BCL antibodies have not been identified.
Methodology: 176 serum samples, from patients diagnosed with HDV in the Ospedale Aggiore Policlinico in Milan, and 30 sera from healthy donors were tested. An indirect immunofluorescence (IIF) on sections of monkey esophagus were realized. Antigen characterization was performed with human and rat epidermal extracts. To confirm the results a capture ELISA has been designed with monoclonal antibodies against recombinant keratin 14 (K14) and keratin 5 (K5).
Results: 49 of the 176 HDV samples (27.8%) have anti-BCL antibodies by IIF and 1 control serum (3.3%). 1 dimension immunoblot on human and rat extracts reveals the presence of a 51 kDa band which, upon sequencing, gave significant identifications for keratins 1, 5, 9, 10, 14 and 16. Only keratins 5 and 14 are present in epidermis BCL, then we design a capture ELISA for K14 and K5 to confirm these antigenic specificities. 114 HDV sera (65.5%) were positive for K14 and 112 HDV sera (64.4%) were positive for K5 and 2 (6.7%) and 1 (3.3%) control sera were positive for K14 and K5 respectively.
Conclusion: The present study has identified K14 and K5 as main target antigens that recognize autoantibodies against BCL. This identification has been confirmed through a capture ELISA with recombinant K14 and K5.

P.C1.06.04
Extra-criteria antiphospholipid antibodies in seronegative antiphospholipid syndrome of central nervous system with small vessel brain lesions

M. A. Estévez[1,2], *A. Molina-Fuentes*[1,2], *A. López-Gómez*[1,2], *M. R. Jiménez*[1,2], *N. Lanio*[1,2], *M. Montes*[1], *M. R. Fuster*[1], *J. Rascón*[1], *S. Tur*[1], *M. Picado*[1], *L. Sáez*[3], *S. Sánchez*[4], *L. Pallarés*[1], *M. R. Julià*[1,2];
[1]Hospital Universitario Son Espases, Palma de Mallorca, Spain, [2]Institut d'Investigació Sanitària Illes Balears (IdISBa), Palma de Mallorca, Spain, [3]Hospital Universitario Miguel Servet, Zaragoza, Spain, [4]Hospital Fundación Alcorcón, Madrid, Spain.

Background. Seronegative Antiphospholipid Syndrome (SNAPS) refers to patients with clinical profile suggestive of APS but persistently negative for antiphospolipid antibodies (aPL) included in APS classification criteria: anticardiolipin (ACA), anti-beta2Glycoprotein I (B2GP) (both IgG or IgM), and lupus anticoagulant.
Patients and methods. We studied 65 patients with small vessel brain lesions (SVBL), MRI and clinical manifestations compatible with APS and negative for aPL included in APS criteria. We also tested 24 autoimmune controls. We performed:
1-ELISA assays for B2GP and ACA (IgA); anti-phosphatidylserine/prothrombin (PS/PT), phosphatidylethanolamine, prothrombin (PT) (IgG, IgM) and anti-annexin V (IgG).
2-Chemiluminiscence assay for antibodies to Domain 1 of B2GP IgG.
Results. We found 13 patients positive for extra-criteria aPL: 2 (3.1%) for B2GP IgA; 4 (6.1%) for PT IgG, 1 of them was low positive; 4 (6.1%) for PT IgM , 2 were low positive for PS/PT
IgM (3.1%) and 1 was low positive for PT IgG and PS/PT IgM (1.5%). Only 3 controls were positive: 1 for PS/PT IgM, 1 for PT IgG and 1 for Annexin V, all of them low positive. Excluding low positive results, we detected 9 patients and 0 controls positive.
Conclusions. The presence of Abs to B2GP (IgA) and to PT (IgG and IgM) allowed us to identify as aPL-positive 13.8% of seronegative SVBL patients presenting MRI and clinical findings compatible with APS. Follow-up of these patients and additional studies will confirm our results.
This work was funded by a grant from the Spanish Society of Internal Medicine (ref: SAFSN-SNCpv).

P.C1.06.05
Pro-inflammatory histidyl-tRNA synthetase-specific CD4$^+$ T-cells are enriched in the lung of patients with inflammatory myopathies and antisynthetase syndrome

A. S. Galindo-Feria[1,2], *I. Albrecht*[1,2], *C. Fernandes-Cerqueira*[1,2], *A. Notarnicola*[1,2], *E. A. James*[3], *J. Herrath*[1,2], *M. Dastmalchi*[1,2], *T. Sandalova*[4,5], *K. Tandre*[6], *L. Rönnblom*[6], *P. Jakobsson*[1,2], *M. Fathi*[7], *A. Achour*[8,9], *J. Grunewald*[10], *V. Malmström*[1,2], *I. E. Lundberg*[1,2];
[1]Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden, [2]Center for Molecular Medicine, Stockholm, Sweden, [3]Benaroya Research Institute at Virginia Mason, Seattle, Washington, United States, [4]Science for Life Laboratory, Department of Medicine Solna, Karolinska Institute, Stockholm, Sweden, [5]Department of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden, [6]Department of Medical Sciences, Rheumatology, Science for Life Laboratory, Uppsala University, Institutet, Karolinska University Hospital, Uppsala, Sweden, [7]Department of Respiratory Medicine and Allergy, Karolinska University Hospital, Stockholm, Sweden, [8]Science for Life Laboratory, Department of Medicine Solna, Karolinska Institute,, Stockholm, Sweden, [9]Department of Infectious Diseases, Karolinska University Hospital, Solna, Stockholm, Sweden, [10]Respiratory Medicine Unit, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.

Objectives To investigate immunity against histidyl-tRNA synthetase (HisRS) in blood and lungs of idiopathic inflammatory myopathy (IIM)/anti-synthetase syndrome (ASS) patients. **Methods** Bronchoalveolar lavage fluid (BALF) cells, and peripheral blood mononuclear cells (PBMCs) from IIM/ASS patients (n=24) were stimulated with full-length HisRS-protein and/or a HisRS-peptide representing a potential T-cell epitope (amino acids 11-23). Controls were cells from sarcoidosis patients (n=16), and HLA-matched healthy subjects (HCs n=18). Antigen-specific activation of CD4$^+$T-cells was assessed by CD40L up-regulation and cytokine expression using flow cytometry. Anti-Jo1 antibodies in serum and BALF were investigated by ELISA. CD3$^+$ T-cells and CD138$^+$ plasma cells were investigated by immunohistochemistry in lung biopsies from IIM and obstructive pulmonary disease patients. **Results** Increased CD40L up-regulation was detected in BALF cells from 2 out of 3 IIM/ASS patients stimulated with HisRS-peptide (median-fold [IQR]: 27 [1-148]) and in PBMCs from 14/18 patients with HisRS-protein (5[2-26]). Elevated CD40L expression in BALF cells stimulated with HisRS-protein and HisRS-peptide was observed in 2/7 sarcoidosis patients; and in 1/12 PBMCs from HLA-DRB1*03-HCs stimulated with HisRS-protein. Activated HisRS-specific T-cells in IIM/ASS displayed a Th1 phenotype in BALF when compared to PBMCs (production of IFN-γ$^+$: 60% vs 10%). Anti-Jo1 antibodies were detected in BALF and germinal center-like structures in lung biopsies of anti-Jo1$^+$ patients. **Conclusions** We report HisRS-reactive CD4$^+$ T-cells in both PBMCs and BALF cells of IIM/ASS patients. This together with anti-Jo1 antibodies in BALF and CD138$^+$ cells in lung tissue indicates that immune activation against HisRS might be triggered in the lungs of anti-Jo1$^+$ IIM patients.

P.C1.06.06
Clinical relevance of the new auto-antibodies in dermatomyositis/polimyositis

D. Garcia-Cuesta[1], *M. Vilches-Moreno*[1], *C. Collantes-Rodriguez*[2], *D. Jimenez-Gallo*[2], *L. Ossorio-García*[2], *M. Linares*[2], *C. Rodriguez*[1];
[1]UGC Hematology, Immunology and Genetics. Hospital Puerta del Mar., Cádiz, Spain, [2]UGC Dermatology. Hospital Puerta del Mar., Cádiz, Spain.

Introduction: Dermatomyositis and polymyositis (PM/DM) are inflammatory myopathies characterized by muscular weakness and inflammation. Some patients also present with characteristic skin changes, interstitial lung disease (ILD) or cancer, among other symptoms. Myositis-specific and myositis-associated autoantibodies (autoAb) have been found in patients. In addition, new specific AutoAb have been included in the diagnostic panels.
Objective: To asses the clinical value of the new PM/DM-specific AutoAb in defining clinical phenotypes in our patients diagnosed with PM/DM.
Materials and methods: All patients with a suspicion of PM/DM admitted to Hospital Puerta del Mar (Cadiz, Spain) for the last 18 months were tested for anti-TIF1-γ, anti-NXP2, anti-MDA5 and SAE-1 AutoAb (New DM/PM-autoAb) by using immunoblot (Euroimmun, Germany). Clinical features were recorded.
Results: New DM/PM-AutoAb were detected in 3 patients. Patient 1 had anti-TIF1-γ AutoAb and showed an exclusive cutaneous phenotype. Neither ILD nor cancers were found. Patient 2 had anti-MDA5 AutoAb and showed a skin rash with progressive ILD. Patient 3 had Anti-NXP2 AutoAb and presented with severe skin changes without ILD or cancer. Patients did not show evident muscular weakness, although muscle enzymes were elevated in patients 2 and 3.
Conclusions: In our patients, anti-MDA5 AutoAb was a good marker for ILD and NXP2 AutoAb indicated severe skin disease. Neither TIF1-γ nor NXP2 were markers for associated cancer in PM/DM. These results support the role of the new DM/PM-AutoAb in defining the clinical phenotype and the prognosis of DM/PM patients.

P.C1.06.07

Frequency of anti-nuclear antibody and anti dsDNA antibodies in subjects of oral addictive habits

M. Kashif[1], *N. Afzal*[1], *S. Minhas*[2], *M. A. Anwar*[1], *F. S. Khan*[1], *S. Jahan*[1];
[1]*University of Health Sciences, Lahore, Pakistan,* [2]*Akhtar Saeed Medical and Dental College, Lahore, Pakistan.*

Background: People with addictive habits are prone to both infectious and non-infectious diseases. Conflicting results have been reported about propensity of these individuals for development of autoimmune diseases. Therefore, a study was planned to determine the frequency of anti-nuclear antibody (ANA) and anti-dsDNA (dsDNA) antibody in the serum of habitual smokers, paan (areca nut) chewers and other oral addictive habits as compared to subjects without such addictive habits.
Methods: Blood samples from 90 subjects (45 with addictive habits and 45 without any addiction) were taken by random sampling after getting written informed consent. Enzyme linked immunosorbent assay (ELISA) was used to test the sera for ANA and anti dsDNA.
Results: One subject in the addictive group had ANA and dsDNA antibodies, whereas in the control group, two subjects had anti dsDNA while none of them had ANA. No significant association of these antibodies was observed between the two groups.
Conclusions: Addictive habits do not predispose the subjects to develop autoimmune diseases.

P.C1.06.08

Functional role of CD83 expressed by Foxp3$^+$ regulatory T cells in the context of inflammatory bowel disease.

C. Koenig*, A. Steinkasserer, M. Lechmann;*
Department of Immune Modulation at the Department of Dermatology, University Hospital Erlangen, Erlangen, Germany.

Inflammatory bowel disease (IBD) is still a significant health problem characterized by chronic and recurrent inflammation of the gastrointestinal tract. Unfortunately, available treatment options for IBD are not satisfactory and therefor, a great need for new and more efficient therapeutic strategies exists. Interestingly, using murine IBD models our group showed that activated Tregs rapidly upregulate CD83 expression and that the application of soluble CD83 ameliorates the clinical symptoms in the DNBS-induced colitis model. To investigate the underlying mechanisms, we generated CD83 conditional KO animals (CD83cKO), whereby CD83 expression has been specifically deleted on Tregs only. Using these animals we investigated in detail whether deletion of CD83 modulates the suppressive function and/or development of Tregs in steady state and in the context of IBD. The clinical impact of CD83 expression on Tregs was monitored using histology, weight loss and endoscopy. Phenotypically, these Tregs were characterized by FACS-, RT-PCR- and CBA. Body weight and health status revealed an aggravated colitis in CD83cKO mice which correlated with a highly increased mortality rate in these mice. In addition, elevated expression of pro-inflammatory mediators and reduced numbers of Foxp3$^+$CD103$^+$ and Foxp3$^+$KLRG1$^+$ Tregs were observed in CD83cKO mice. In addition, we are currently evaluating the therapeutic potential of sCD83 as new strategy for the treatment of IBD by using the murine transfer colitis model.
Our results provide first detailed insights into the mechanistic role of CD83 expressed on Tregs and represent the basis for the development of new specific therapies for patients suffering from autoimmune disorders.

P.C1.06.10

β-Catenin signaling in CD11c$^+$myeloid cells regulates immune homeostasis in the intestine

C. Kurz[1], *A. Brand*[1], *F. Bauer*[1], *A. Jiang*[2], *I. Mellman*[3], *J. Ober- Blöbaum*[1], *B. E. Clausen*[1];
[1]*Institute of Molecular Medicine, Mainz, Germany,* [2]*Department of Immunology, Roswell Park Cancer Institute, Buffalo, NY 14263, United States,* [3]*Research Oncology, Genentech, South San Francisco, CA 94080, United States.*

Introduction: Chronic inflammation in the intestine arises from a loss of tolerance towards harmless environmental antigens like nutrients or commensal bacteria. Myeloid antigen-presenting cells (APC), including macrophages (MØ) and dendritic cells (DC), play essential roles balancing immunity and tolerance. β-Catenin is the central component of the canonical Wnt signaling pathway and has previously been demonstrated to promote a tolerogenic DC phenotype *in vitro*. Hence, CD11c-specific β-catenin deficiency aggravates disease in a mouse model of DSS-induced colitis, suggesting that β-catenin is a key regulator of myeloid cell function *in vivo*. Whether β-catenin in APC is able to attenuate intestinal inflammation remains elusive.
Methods: To further investigate the role of CD11c-specific β-catenin signaling in the regulation of intestinal immune homeostasis, we generated mice with either a deletion (CD11c-βcat^{DEL}) or expressing a stabilized form of β-catenin (CD11c-βcat^{EX3}). Subsequently, these mice were analyzed in the steady-state as well as in DSS-induced colitis.
Results: In steady-state mesenteric lymph nodes (mLN), CD11c-βcat^{EX3}mice exhibited higher numbers of DC and regulatory T cells (Tregs) as compared to controls, whereas DC and Treg numbers in mLN of CD11c-βcat^{DEL}mice remained unchanged. Moreover, CD11c-βcat^{EX3}mice were less susceptible to DSS-induced colitis accompanied by increased numbers of Foxp3$^+$Tregs.
Conclusion: Our data indicate that activation of β-catenin signaling in CD11c$^+$myeloid cells supports their tolerogenic function via the induction of Tregs. In ongoing experiments we are dissecting the molecular mechanism(s) by which β-catenin enables distinct MØand DC subsets to regulate intestinal immune homeostasis in the steady-state and during inflammation.

P.C1.06.11

A deep analysis of the intestinal immune cell compartment in dextran sodium sulfate (DSS) induced colitis

M. Letizia[1,2,3], *Y. Rodriguez Sillke*[1], *F. Schmidt*[1], *R. Glauben*[1], *C. Weidinger*[1], *B. Siegmund*[1];
[1]*Charité, Berlin, Germany,* [2]*Humboldt-Universität zu Berlin, Berlin, Germany,* [3]*ZIBI Graduate School Berlin, Berlin, Germany.*

Crohn's disease is characterized by epithelial barrier breaches and a subsequent translocation of bacteria from the intestinal lumen into the adjacent mesenteric fat, inducing the hyperplasia of adipose tissue as well as the recruitment of various immune cells. However, the functional role of mesenteric fat in intestinal immunity and the immunologic imprinting occurring upon epithelial barrier defects is currently unknown. One of the most used mouse model of experimental colitis employs DSS treatment. Although the immune cell composition of colon lamina propria has been investigated, little is known about DSS induced changes within the mesenteric fat. Therefore, we analyzed the immune cell compartment of colon lamina propria in comparison with the mesenteric fat compartment. C57BL/6 mice were fed 2.5% DSS in their drinking water for 5 days to induce acute colitis or 1.5% DSS for 5 days followed by 7 days water in 4 cycles to induce chronic colitis. Immune cells were isolated from mesenteric fat, gonadal fat, mesenteric lymph nodes and lamina propria and analyzed by flow cytometry or mass cytometry. Our data give for the first time a comprehensive characterization of immune cells in colon lamina propria and mesenteric fat in DSS induced colitis and suggest an immune-modulatory function of mesenteric fat in chronic but not in acute intestinal inflammation. Further analyses are needed in order to assess whether the DSS-induced colitis model is able to mimic the role of mesenteric wrapping fat in Crohn's disease adequately.

P.C1.06.12

Induction of antibodies to citrullinated protein antigens (ACPAs) by stressed neutrophils

T. Li*;*
Karolinska Institutet, Stockholm, Sweden.

There are very interesting findings published by Mariana J. Kaplan showing that stressed neutrophils can induce mitochondrial ROS, activation of PAD4 and the formation of neutrophil extracellular traps (NETs). They have also shown that NETs containing citrullinated peptides are internalized by fibroblast-like synoviocyte (FLS) through a RAGE-TLR9 pathway, promoting FLS inflammatory phenotype and their up-regulation of major histocompatibility complex (MHC) class II. Once internalized, arthritogenic NET peptides are loaded into FLS MHC class II and presented to antigen-specific T cells. HLA-DRB1*04:01 transgenic mice immunized with mouse FLS loaded with NETs develop antibodies specific to citrullinated forms of relevant autoantigens implicated in rheumatoid arthritis (RA) pathogenesis as well as cartilage damage. These results implicate FLS as notable mediators in RA pathogenesis, through the internalization and presentation of NET citrullinated peptidesto the adaptive immune system, leading to pathogenic autoimmunity and cartilage damage. Therefore, it will be interesting to study whether proteins from stressed neutrophils can be used to immunize the humanized mouse strains to induce ACPA production. To link periodontal infection to ACPA, we will use leukotoxin A (LtxA), a toxin from Aggregatibacter actinomycetemcomitans (Aa), which has been shown to induce hypercitrullination in host neutrophils. We have cultured FLS in vitro successfully and verified that these FLS have the ability to present antigens to CD4$^+$ T cells. Next we plan to immunize DRB1*0401. Ncf1*/* mice with cit-peptides loaded FLS and detect whether can induce the production of ACPAs and RA.

P.C1.06.13

Compartmentalized tissue adaptation of human colonic CD4⁺ T cells

L. Lutter[1], *D. P. Hoytema van Konijnenburg*[2], *J. J. ter Linde*[1], *A. Petrelli*[3], *N. R. Lansu*[1], *J. ten Hove*[1], *M. van der Wal*[1], *V. Meij*[1], *H. H. Fidder*[1], *M. Mokry*[1], *B. Oldenburg*[1], *F. van Wijk*[1];
[1]*University Medical Centre Utrecht, Utrecht, Netherlands,* [2]*Rockefeller University, New York, United States,* [3]*IRCCS San Raffaele Scientific Institute, Milan, Italy.*

The mucosal barrier of the gut is home to numerous intraepithelial (IE) and lamina propria (LP) T cells adapted to the local environment in order to maintain homeostasis. Recent work on tissue T cells has focused on CD8⁺ "tissue resident memory cells" (Trm); however, the human colon also harbors different, less characterized, CD4⁺ T cell populations. Tissue resident T cells have been suggested to play important roles in relapsing-remitting inflammatory diseases including inflammatory bowel disease; hence, we FACS-sorted human CD4⁺ IE and LP T cell populations for RNA sequencing. We show that LP CD4⁺CD8aa⁺ resemble the murine cytotoxic CD4⁺CD8aa⁺ IE T cells (e.g. expression of GZMA, GZMB, PRF1, and IFNG), and LP CD4⁺CD25⁺ cells are similar to classical regulatory T cells. CD4+CD25+ are similar to CD4⁺CD8aa⁺ IE T cells with upregulation of immune regulatory pathways, and both populations are quite different from their LP counterparts.
Furthermore, IE and LP CD4⁺CD8aa⁺ possess hallmarks of Trm cells, but do not fully recapitulate the phenotype as described in the literature for CD8⁺ Trm. During inflammation, flow cytometry analyses showed an increased IE and LP CD4⁺:CD8⁺ ratio compared to remission values, as well as an increase in CD4⁺ T cells expressing lower or no amounts of CD69. Additionally, we found a trend towards relative decreased CD4⁺CD8aa⁺ and increased CD4⁺CD25⁺ T cell presence both in the LP and IE compartment during inflammation. This loss of compartmentalized tissue adaptation of human colonic CD4⁺ T cells can have potential detrimental effects on barrier maintenance and continued inflammation.

P.C1.06.14

Immunological findings in patients with recurrent aphthous stomatitis

J. Petanova[1,2], *R. Cermakova*[3], *M. Libanska*[3], *J. Bartova*[3], *Z. Jiraskova Zakostelska*[4], *Z. Stehlikova*[4], *H. Tlaskalova-Hogenova*[4], *L. Izakovicova-Holla*[5,6], *S. Slezakova*[5,6], *P. Borilova Linhartova*[5,6], *P. Kuklínek*[7];
[1]*General University Hospital in Prague, Institute of Immunology and Microbiology, Prague 2, Czech Republic,* [2]*Charles University, Institute of Immunology and Microbiology, Prague, Czech Republic,* [3]*General University Hospital in Prague, Institute of Clinical and Experimental Dental Medicine, Prague 2, Czech Republic,* [4]*Institute of Microbiology, The Czech Academy of Sciences, Prague, Czech Republic,* [5]*Clinic of Stomatology, Institution Shared with St. Anne's Faculty Hospital, Faculty of Medicine, Masaryk University, Brno, Czech Republic,* [6]*Department of Pathophysiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic,* [7]*Department of Clinical Immunology and Allergology, Institution Shared with St. Anne's Faculty Hospital, Faculty of Medicine, Masaryk University, Brno, Czech Republic.*

The etiology of recurrent aphthous stomatitis is not yet clear, immunopathologic states like autoimmunity, allergy or deficiency are mentioned either. We analyzed the immunological status of 73 patients with recurrent aphthous stomatitis (28 men, 45 women, aged 18-72 years). The following parameters were studied: humoral immunity (serum concentration of immunoglobulin IgG, IgA, IgM, IgE, IgG subclasses, complement components C3 and C4, acute phase protein CRP, selected autoantibodies), cellular immunity (total number of lymphocytes, T lymphocytes subpopulations, B lymphocytes, natural killer cells). The immunological parameters in cellular immunity were in normal ranges. In humoral immunity we found in 13 patients slightly decreased or increased serum concentrations of IgG (4), IgA (6), IgM (3), elevated IgE levels occurred in 14 patients. Antinuclear antibodies were slightly positive or positive in 15 patients (21%). Eighteen (38%) of 48 patients had slightly positive antibodies to desmogleins. The clinical periodontal finding and in some cases mucosal biopsy with histopathological findings excluded autoimmune blistering diseases in all tested patients. We found high inter-individual variability in anamnestic and laboratory data in the group of patients with recurrent aphthous stomatitis. The positivity of antibodies to desmogleins is not specific, positive results can be found in different inflammatory mucosal or cutaneous states. More specific tests have to be performed together with specialized periodontal clinical findings. The work was supported by Ministry of Health of the Czech Republic, grant nr.15-29336A.

P.C1.06.15

The impact of food antigens on the intestinal homeostasis and inflammation in inflammatory bowel disease

Y. Rodríguez Sillke[1,2], *U. Steinhoff*[3], *M. Schumann*[1], *C. Bojarski*[1], *D. Lissner*[1], *F. Branchi*[1], *B. Siegmund*[1], *R. Glauben*[1];
[1]*Charité - Universitätsmedizin Berlin, Medical Department (Gastroenterology, Infectious Diseases, Rheumatology) Campus Benjamin Franklin, Berlin, Germany,* [2]*Institute of Nutrition, University of Potsdam, Nuthetal, Germany,* [3]*Institute for Medical Microbiology and Hygiene, University of Marburg, Marburg, Germany.*

One of the hallmarks of inflammatory bowel diseases (IBD) is a dysregulation of the intestinal immune system. Although nutritional therapy with the elemental diet proves to be effective, little is known about its mechanism. Murine data indicate that food antigens induce an activation and subsequent apoptosis of the CD4⁺ T-cells in the Peyer's Patches (PP) thus maintaining the healthy balance of the mucosal immune system.
PP T-cells were characterized for patients of Crohn´s disease (CD) and Ulcerative colitis (UC) as well as healthy controls. Gluten served as a model food antigen. Thus gluten activated CD4⁺ T-cells in the peripheral blood of these patients were analysed by a magnetic enrichment of CD154⁺ cells and a subsequent cytometric antigen-reactive T-cell analysis (ARTE technology).
CD4⁺ T cells isolated from PP of CD patients revealed a significantly reduced apoptotic rate compared to UC patients and healthy controls. This was accompanied by an increased expression of the survival marker Bcl-2. Further characterization identified an up-regulation of FoxP3 as marker for regulatory T-cells, as well as the activation marker, Helios in CD patients. Moreover, there was a higher frequency of gluten antigen-specific T-cells (CD4⁺CD154⁺) in the peripheral blood of CD patients expressing pro-inflammatory cytokines.
The decreased apoptosis in parallel to an enhanced survival of CD4⁺ T-cells in PP of CD patients suggests a pathological T-cell hyperactivation followed by a disturbed immune homeostasis. Additionally, our data provide evidence that the IBD dependent disruption of the intestinal barrier suffices to induce food-antigen specific pro-inflammatory T-cells in the periphery.

P.C1.06.16

Anti-GP2 antibodies in blood and feces of children with inflammatory bowel diseases.

A. Toptygina[1,2], ***E. Semikina***[3,4], *S. Petrichuk*[5];
[1]*G.N.Gabrichevsky Research Institute for Epidemiology and Microbiology, Moscow, Russian Federation,* [2]*Lomonosov Moscow State University, Moscow, Russian Federation,* [3]*Federal State Autonomous Institution, Moscow, Russian Federation,* [4]*Pirogov Russian National Research Medical University, Moscow, Russian Federation,* [5]*Federal State Autonomous Institution "National Medical Research Center of Children's Health", Moscow, Russian Federation.*

Inflammatory bowel diseases (IBD), such as Crohn's disease (CD) and ulcerative colitis (UC), are associated with considerable morbidity and reduced quality of life. Anti-glycoprotein2 (GP2) antibodies were found in the blood of CD-patients. The aim was to examine the anti-GP2 antibody levels in blood and feces of CD and UC-patients. Serum and coproextract probes from 110 children (64 boys and 46 girls) aged 12.3(2.6-17.9) years old were examined: 36 CD-patients, 30 UC-patients, and 44 control patients with intestinal dysbiosis (DB). IgG and IgA anti-GP2 antibodies were tested by ELISA. Cut-off calculated for children's IgG anti-GP2 antibodies for IBD versus non-IBD was 13.8U/ml (sensitivity 63.2%, specificity 100%). Cut-off for IgA was 5.63U/ml (sensitivity 60.5%, specificity 78.8%). Among 36 CD-patients 10(27.7%) had IgG and 15(41.7%) had IgA anti-GP2 antibodies. In 30 UC-patients 5(16.6%) had IgG and 10(33.3%) had IgA anti-GP2 antibodies. None of 44 DB-patients had IgA and only 1(3.3%) had IgG (15.5U/ml). The level of anti-GP2 antibodies in the serum of CD-patients was significantly higher in comparison to UC-patients ($p<0.05$) and control group ($p<0.01$). The feces levels of anti-GP2 IgG were elevated in CD(23.5U/ml) and UC(20.45U/ml) patients versus 1.99U/ml in DB ($p<0.01$). No differences in anti-GP2 IgA levels between IBD-patients and DB were found. However IBD-patients had higher level of sIgA, and the ratio of anti-GP2IgA/sIgA was significantly lower in CD(0.326) and UC(0.327) than in DB(2.332) patients ($p<0.01$). Differences in anti-GP2 IgG and IgA profiles between IBD and DB patients reflect the peculiarities of disease immunopathogenesis.

P.C1.06.17

***In situ* expression of IgA and IgG in intestinal mucosa of algerian patients with inflammatory bowel disease**

R. Toumi[1], *I. Soufli*[1], *S. Ait Younes*[2], *C. Touil-Boukoffa*[1];
[1]*University of Sciences and Technology Houari Boumedienne, Algiers, Algeria,* [2]*Anatomic Pathology Service, Mustapha Pacha Hospital, Algiers, Algeria, Algiers, Algeria.*

The intestinal mucosa is home to the largest population of Antibody-secreting plasma cells. The antibodies released by these cells constitute a first line of protection but could also be involved in autoimmune processes. Although the role of immunoglobulins in triggering inflammatory bowel disease (IBD) has not yet been established, there is many data to support this hypothesis. IBD including Crohn diseases (CD) and Ulcerative colitis (UC) is chronic multi-factorial disorder affecting the gastrointestinal. In this study, we investigated by immunohistochemical study the expression of IgA and IgG in the intestinal mucosa of Algerian patients with CD and UC. Our results revealed the presence of a significantly high number of IgA + and IgG + cells for both categories of patients compared with healthy mucosa ($p<0.05$). The analysis of IgA and IgG expression at different regions of intestine of the patients did not show a significant difference. However our data, showed the presence of high number of IgA+ cells in the colonic mucosa of patients with CD in comparison with UC. This result could be explained by the strong expression of NOS-2 and TNF-α in MC that maintain IgA-producing plasma cells in the intestine. Moreover, a slight predominance of IgG + cells in the colonic mucosa of patients with CD was observed. This can be explained by the nature of the immune profile characterizing both pathologies. In summary, the current study provides additional evidence for the Involvement of plasma cells secreting IgG and IgA in the pathophysiology of IBD.

P.C1.06.18

HMGB1 released from intestinal epithelia damaged by cholera toxin adjuvant contributes to activation of mucosal DCs and induction of intestinal CTLs and IgA

***A. Wakabayashi**, M. Shimizu, E. Shinya, H. Takahashi;*
Nippon Medical School, Tokyo, Japan.

Oral administration of OVA plus cholera toxin (CT), but not the CTA or CTB subunit, induces OVA-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) in intraepithelial lymphocytes (IELs). The intestinal OVA-specific CTLs were not induced in $CD11c^+$ dendritic cell (DC)-depleted CD11c-DTR mice. $CD8^+CD103^+CD11c^+CD11b^-$ DCs and $DCIR2^+CD103^+CD11c^+CD11b^+$ DCs were distributed in the intestinal lamina propria and mesenteric lymph nodes, both DC subsets expressed DEC-205, and the expression of costimulatory molecules such as CD80 and CD86 was enhanced in both DC subsets after oral administration of intact CT but not the CTA or CTB subunit. Intestinal DCs activated by the oral administration of OVA plus CT cross-presented OVA antigens, and DCs that captured OVA antigen through DEC-205, but not DCIR2, could cross-present antigen. We found that oral administration of intact CT, but not the CTA or CTB subunit, enhanced cell death, cytoplasmic expression of high mobility group box 1 protein (HMGB1) in epithelial cell adhesion molecule $(EpCAM)^+CD45^-$ intestinal epithelial cells (IECs) and HMGB1 levels in fecal extracts. HMGB1 dose-dependently enhanced the expression of CD80 and CD86 on DCs *in vitro*, and intravenous or oral administration of glycyrrhizin, an HMGB1 inhibitor, significantly suppressed activation of mucosal DCs and induction of intestinal OVA-specific CTLs and IgA by oral CT administration. These results showed that oral administration of intact CT triggers epithelial cell death in the gut and the release of HMGB1 from damaged IECs and that the released HMGB1 may mediate activation of mucosal DCs and induction of CTLs and IgA in the intestine.

P.C1.06.19

Alterations in Immune cell subsets in patients with chronic silicosis caused by artificial quartz agglomerates

*G. Jimenez-Gomez[1], A. Hidalgo-Molina[1], A. Perez-Alonso[2], J. M. Morales-Morales[3], J. A. Cordoba Doña[4], A. Leon Jimenez[5], **A. Campos-Caro**[1];*
[1]Hospital Universitario Puerta del Mar, Cádiz, Spain, [2]Preventive Medicine Service, Cádiz, Spain, [3]Hospital Universitario Puerto Real, Cádiz, Spain, [4]Public Health Service, Cádiz, Spain, [5]Hospital Universitario Puerta del Mar, Cadiz, Spain.

Background: Silicosis produced by Artificial Quartz Agglomerates (AQA) evolves more aggressively than the classical form of miners. This entity is emerging worldwide and a significant group of cases has been detected in the province of Cádiz (Spain) in recent years. The role of the cellular immune response in the pathogenesis of silicosis by AQA has not been previously studied. **Objective:** to analyse cell populations present in peripheral blood from patients with silicosis by AQA and and compare them with the ones obtained from healthy volunteers. **Methods:** 48 patients diagnosed with silicosis by AQA and 18 healthy controls were studied. The blood cells populations were quantified by flow cytometry. **Results:** No differences were found in the total number of leukocytes or granulocytes. However, a significant increase in monocytes cell number and a clear lymphocytopenia were observed in the blood from patients compared to healthy controls. Almost all the lymphocyte subsets studied - B lymphocytes, T lymphocytes and NK cells ("Natural Killers") - were decreased compared to healthy controls. Particularly, a significant decrease in the total cell number was observed in the following subsets: memory B lymphocytes; T-helper lymphocytes, naïve and memory T-lymphocytes, T-regulatory lymphocytes and $CD56^{Brigth}$ NK cell subpopulations. However, there was a significant increase in the TH1 and TH17 subsets as well as in plasma cells in patients. **Conclusions:** These alterations in blood cell populations could reflect different states of inflammatory and fibrotic activity in these patients.

P.C1.07 Maintenance and local regulation of tissue specific immunity - Part 7

P.C1.07.02

G protein-coupled receptor 15 is associated with smoking and relapsing remitting multiple sclerosis

***C. Ammitzbøll**, M. R. von Essen, L. Börnsen, E. Petersen, O. McWilliam, H. B. Søndergaard, F. Sellebjerg;*
Danish Multiple Sclerosis Center, Copenhagen, Denmark.

Background: Smoking is an established risk factor associated with autoimmune diseases including multiple sclerosis (MS). We have studied the effect of smoking on circulating immune cells and found *GPR15* mRNA expression upregulated in healthy smokers and patients with relapsing remitting MS (RRMS). The protein expression of GPR15 was related to smoking but not RRMS. *GPR15* encodes a G protein-coupled receptor on lymphocytes, which together with the recently identified GPR15L, is involved in immune homeostasis in the skin and colon.
Objective: To study GPR15L and GPR15 expressing cells in the cerebrospinal fluid (CSF) of smokers and patients with RRMS.
Results: By flow cytometry we found higher frequencies of GPR15+ T cells in CSF compared with blood. In smokers, blood- and CSF frequencies of GPR15+ T cells were significantly increased and were in the CSF associated with chemokine receptors CCR6 and CXCR3 compared with GPR15- T cells. ELISA analyses showed lower concentrations of GPR15L in CSF than in blood and concentrations were unaffected by smoking or RRMS. In patients with RRMS, GPR15L were positively correlated with CD4+GPR15+CCR6+CXCR3+ T-cells, myelin basic protein (MBP) and functional disability measured by the expanded disability status scale (EDSS).
Conclusion: In the present study we suggest a novel cell type in MS pathogenesis, linked to smoking. Smoking increases circulating GPR15+ T cells with CNS migration potential (CCR6 and CXCR3 expression). In patients with RRMS, these cells (from the CD4+ T cell population) are associated with GPR15L in the CSF, damage of the myelin sheaths and functional disability.

P.C1.07.03

The HLA-DR3 peptide repertoire in epithelial cells versus the conventional antigen processing

***Y. Arribas**[1,2], J. A. Collado[1], R. Farriol[1,2], V. Casas[2], M. Carrascal[2], D. Jaraquemada[1];*
[1]Cellular Immunology, Institute of Biotechnology and Biomedicine, Cerdanyola del Vallès, Barcelona, Spain, [2]CSIC/UAB Proteomics Laboratory, IIBB-CSIC, Cerdanyola del Vallès, Barcelona, Spain.

HLA-DR3 (HLA-DR17, DRB1*0301/DRA1*0101) is an HLA-DR allele associated to a high number of autoimmune diseases, including type 1 diabetes or Graves' disease. Previous work on HLA-DRB1*0301-positive Graves' thyroid tissues revealed the presence of peptides from tissue-specific antigens in the HLA-DR-associated repertoire. The peptide repertoires of human $DR3^+$ spleen samples were then processed and compared with thyroid tissue. The results showed a clear tissue-specific bias in the spleen repertoire, where a large frequency of peptides derived from blood borne proteins. In addition, the overall affinity of the peptides from the spleen was higher than that of thyroid peptides. In order to identify possible mechanisms of differential HLA-II processing in $MHC\text{-}II^+$ epithelial cells versus canonical APCs, peptide repertoires were studied from a human HLA-DR3, -Ii and -DM transfected rat insulinoma epithelial cell line compared with a homozygous $DR3^+$ lymphoblastoid B cell line. The results showed little differences between both repertoires in parameters such as affinity or hydrophobicity. Tissue specificity was evident in the epithelial repertoire. We identified several peptides derived from proteins restricted to neuroendocrine tissues, including T1D autoantigen ICA512 or autoimmune uveitis-related calpain-5. In contrast, the peptides found in EBV were mostly derived from ubiquitous proteins. Interestingly, a high frequency of cytosolic-degraded peptides was found in the DR3 repertoires, both from epithelial cells, B-LCLs and spleen cells, to a maximum of 40% in the spleen samples. This contrasts with the B-LCL repertoires associated to most HLA-DR alleles, which usually contain around 20% of such peptides.

P.C1.07.05

Rapid isolation of functional ex vivo human skin resident memory T cells

***W. Du**[1], C. Cendon[1], A. Schulz[1], E. Zhang[2], J. Bodo[3], H. Chang[1], A. Radbruch[1], J. Dong[1];*
[1]German Rheumatism Research Center Berlin, Berlin, Germany, [2]Sankt Gertrauden Krankenhaus, Berlin, Germany, [3]Plastische und Ästhetische Chirurgie, Berlin, Germany.

Introduction
In mice, skin-resident memory T (T_{RM}) cells have been shown to provide rapid local protection. To further dissect human skin T_{RM} cells, various isolation approaches have been applied, e.g. EDTA isolation, collagenase P digestion, and skin explants. However, these protocols either suffer from low yield or require long ex vivo culture periods. We established a modified collagenase IV digestion protocol for rapid isolating high yield viable T_{RM} cells while preserving intact epitopes of interest.
Materials and Methods
Skin samples were obtained from healthy donors under plastic surgeries. Subcutaneous fat were removed and the remaining tissue was minced and incubated in digestion medium at 37°C for 6 hours. Subsequently, the digested skin fragments were dissociated and the cell suspension was filtered and stained with various surface markers. Viable cells were counted; the expressions of CD45 and resident memory T cell markers were analyzed. In parallel, this protocol was compared with other protocols such as whole skin dissociation and collagenase P digestion.
Results and Conclusions
In terms of yield and cell viability, the modified collagenase IV digestion protocol resulted in at least 1.5 times more T cells per cm^2 with relatively higher viability than other protocols. Moreover, this modified protocol could well preserve critical surface marker expressions (e.g. CD4, CD8 and CD69), as opposed to other protocols. Therefore, the modified collagenase IV digestion protocol is suitable for further functional assays which acquire relative high amount of viable skin T cells, providing an opportunity for better understanding of human skin T_{RM} cells.

P.C1.07.06

FOXO1-activity controls CD8 T cell effector function and prevents liver immune pathology during viral hepatitis and non-alcoholic steatohepatitis

M. Dudek;
Institute of Molecular Immunology and Experimental Oncology, Munich, Germany.

Introduction: Upon antigen-recognition, cytotoxic-T-lymphocytes (CTLs) eliminate infected cells. Cellular co-regulation of metabolism and immunity is known to influence effector function, but the mechanisms controlling organ-specific immunity in tissues rich in nutrients such as liver remained unclear. Here we identify FOXO1-activity in CTLs as critical regulator of their metabolic activation during liver disease states. **Material and Methods:** Extracellular flux analysis, cytokine expression, cytotoxicity assays were performed to study CTL co-regulation of metabolism and immunity and its dependence on FOXO1. Murine models of viral hepatitis and non-alcoholic steatohepatitis (NASH) were used to explore FOXO1-dependent T cell immunopathology. **Results:** We previously discovered that FOXO1 was required for memory CD8 T cell-differentiation through cross-priming liver-sinusoidal-endothelial-cells by downsizing cell metabolism. Here we report that, FOXO1-activity also controlled metabolism in effector CTLs. Upon FOXO1-inhibition, CTLs showed augmented metabolic activity, increased gene-expression of nutrient transporters, augmented nutrient-uptake, upregulation of IFN-γ, GzmB and FasL and enhanced effector-functions. Adoptive-transfer of FOXO1-inhibited virus-specific CTLs into mice with viral infection of the liver caused liver immunopathology but failed to control viral infection demonstrating dysbalance between antigen-recognition and execution of effector function. In NASH, hepatic CTLs showed decreased FOXO1-levels and increased cytotoxic potential. Since RNA sequencing did not reveal presence of particular hepatic CTL-clones in NASH, we assume that increased CTL metabolic activation in absence of FOXO1-control caused hepatic immunopathology. **Conclusion:** Our results provide evidence for a critical role of FOXO1 in controlling metabolic CTL-activation required to maintain tissue homeostasis and prevent immunopathology but also to allow for functional tissue immune-surveillance.

P.C1.07.07

The regulation of ZC3H12A and ZC3H12B expression during sterile neuroinflammation

A. Kasza[1]*, D. D. Biswas*[2]*, A. S. Gupta*[2]*, M. Wawro*[1]*, T. Kordula*[2]*;*
[1]*Faculty of Biochemistry, Biophysics and Biotechnology, Cracow, Poland,* [2]*Virginia Commonwealth University, Richmond, United States.*

During sterile neuroinflammation, microglia and astrocytes become activated and release a plethora of pro-inflammatory cytokines such as IL-1β and IL-6. We asked whether activation of astrocytes also triggers a feed-back mechanism, which depends on the synthesis of RNases than degrade pro-inflammatory transcripts. We focused on the regulation of expression of two proteins from ZC3H12 family, namely ZC3H12A/MCPIP1 and ZC3H12B/MCPIP2. The amount of mRNA for Zc3h12a and Zc3h12b was analyzed in the spinal cords of LPS-treated mice as well as during experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis. In both experimental models, the level of Zc3h12a was increased whereas the level of Zc3h12b was decreased. Similarly, treatment of mouse microglia and astrocytes with IL-1β or LPS or human astrocytes with IL-1β, elevated expression of ZC3H12A. However, pro-inflammatory stimuli did not change the level of ZC3H12B. Moreover, these ZC3H12 family members were actively modulating the course of neuroinflammation. Knock-down of ZC3H12A or ZC3H12B up-regulated expression of IL-1β and IL-6 in IL-1-activated astrocytes. Thus, mechanisms, which induce expression of pro-inflammatory cytokines, also activate expression of ZC3H12A, which elicits a feed-back mechanism by regulating pro-inflammatory transcripts turn-over. Regulation of ZC3H12B activity during neuroinflammation remains elusive and needs further investigation.
FUNDING: This work was supported by grant from the Polish National Science Center UMO-2014/15/B/NZ2/03379 (to A.K.) and the Polish-U.S. Fulbright Commission (to A.K.)

P.C1.07.08

TxT-induced MDSCs alter the early systemic pro-inflammatory response and inhibit the antigen-specific T-cell proliferation

Y. Hüsecken[1]*,* ***M. Kustermann***[1]*, M. Huber-Lang*[2]*, K. Debatin*[1]*, G. Straußß*[1]*;*
[1]*Department of Pediatric and Adolescent Medicine, 89075 Ulm, Germany,* [2]*Department of Trauma Surgery, Hand, Plastic and Reconstructive Surgery, 89075 Ulm, Germany.*

Introduction: Trauma activates a strong inflammatory immune response, which is counterbalanced by immunosuppression characterized by an impaired adaptive immune response. Inflammation triggers the induction of myeloid-derived suppressor cells (MDSCs) a heterogeneous population of immature myeloid cells, which suppress various T-cell functions. Thus, induction of MDSCs after an experimental blunt chest trauma (TxT) and their subsequent influence on innate and adaptive immunity was investigated.
Methods: C57BL/6 mice underwent a blast wave to induce TxT. At 6h up to 5d after TxT, increase in MDSCs in lung and spleen and their suppressive capacity in MLR and in vivo after SEB activation was determined. Alterations in the secretion of cytokines and chemokines caused by TxT-induced MDSCs were analyzed in bronchoalveolar lavage (BAL) fluid and blood serum samples.
Results: In TxT-animals, MDSC numbers were preferentially enhanced in the lung until 48h after trauma, while total cell numbers and lymphocyte composition in spleen and lung were not affected. Although, no strong increase in MDSC numbers was detected, splenic MDSCs isolated after TxT suppressed the proliferative capacity of antigen-stimulated T-cells in vitro. Moreover, trauma-induced MDSCs inhibited T-cell proliferation of SEB activated T-cells in vivo and they supported the production of Th1-associated cytokines. TxT-induced MDSCs decreased the systemic levels of IL-6, G-CSF and MCP-1 but didn´t substantially influence the expression of pro-inflammatory factors in BAL fluid.
Conclusions: Thus, our results indicate that TxT promotes the induction of T-cell suppressive MDSCs, which might contribute to trauma-induced immunosuppression.

P.C1.07.09

Development of tissue-resident mucosa-associated invariant T (MAIT) cells in human renal fibrosis and chronic kidney disease (CKD)

B. M. P. Law[1,2]*, R. Wilkinson*[1]*, X. Wang*[1]*, K. Kildey*[1]*, K. Giuliani*[1]*, K. Beagley*[2]*, H. Healy*[1]*, A. J. Kassianos*[1,2]*;*
[1]*Royal Brisbane and Women's Hospital, Brisbane, Australia,* [2]*Queensland University of Technology, Brisbane, Australia.*

MAIT cells are a specialised lymphocyte population associated with chronic inflammatory disorders in peripheral tissues. To date, MAIT cell research has focused primarily on mucosal tissue, with limited studies on non-mucosal organs such as kidneys. In this study, we evaluated MAIT cells in native human kidneys with tubulointerstitial fibrosis, the pathological hallmark of CKD. MAIT cells were identified, enumerated and phenotyped from human kidney tissue by multi-colour flow cytometry. Localisation of MAIT cells were performed by immunofluorescence microscopy. MAIT cells and human primary proximal tubular epithelial cells (PTEC) were cultured under hypoxic (1% O2) conditions to examine mechanistic tubulointerstitial interactions. We detected significantly elevated numbers of MAIT cells (TCR-Vα7.2+ CD161++) in diseased biopsies with interstitial fibrosis compared with diseased biopsies without fibrosis and healthy kidney tissue. The increased numbers of MAIT cells correlated significantly with loss of kidney function (eGFR). MAIT cells in fibrotic biopsies expressed development markers (IL-7Rα, IL-18Rα), activation receptor (NKG2D), extravasation marker (CD44) and tissue-resident markers (CD69, CD103, and CD49a). Immunofluorescent staining of fibrotic kidney tissue localised the accumulation of MAIT cells within the tubulointerstitial compartment, adjacent to PTEC. Notably, PTEC under in vitro pro-fibrotic/hypoxic conditions up-regulates tissue-resident markers CD69 on MAIT cells. We provide the first characterisation of MAIT cells in human kidney tissue. Collectively, our data suggest that human MAIT cells are retained as tissue-resident lymphocytes and are positioned to contribute the fibrosis process via complex interactions with PTEC. Further dissection of kidney MAIT cells offers a novel pathway of disrupting the mechanisms of CKD.

P.C1.07.10

***Eomes*controls the development of Th17-derived (non-classic) Th1 cells during chronic inflammation**

A. Mazzoni[1]*, L. Maggi*[1]*, F. Siracusa*[2]*, M. Ramazzotti*[1]*, M. Rossi*[1]*, V. Santarlasci*[1]*, G. Montaini*[1]*, M. Capone*[1]*, B. Rossettini*[1]*, R. De Palma*[3]*, A. Kruglov*[2]*, H. Chang*[2]*, R. Cimaz*[1]*, E. Maggi*[1]*, S. Romagnani*[1]*, F. Liotta*[1]*, L. Cosmi*[1]*, F. Annunziato*[1]*;*
[1]*University of Florence, florence, Italy,* [2]*German Rheumatism Research Center, Berlin, Germany,* [3]*University of Campania, Naples, Italy.*

Th17 cells are a highly plastic cell subset that can be easily directed towards the Th1 phenotype in *in vitro* and also *in vivo* during inflammation. Instead, there are more concerns regarding the opposite plasticity (i.e. from Th17 to Th1). We show here that ectopic ROR-γt expression can restore or initiate IL-17 expression by non-classic (Th17-derived) or classic Th1 cells, respectively, while common pro-Th17 cytokine cocktails are ineffective. We found that the stability of the Th1 phenotype is at least partially due to the presence of a molecular machinery governed by the transcription factor Eomes, which promotes IFN-γ secretion and inhibits ROR-γt and IL-17. By using a mouse model of T cell-dependent colitis we demonstrate that Eomes controls non-classic Th1 cell development also *in vivo* and promotes their pathogenic potential. Eomes expression associates to a highly inflammatory phenotype also in humans. Indeed, it favors the acquisition of a cytotoxic signature, and promotes the development of IFN-γ$^+$GM-CSF$^+$ cells that have been described to be pathogenic in chronic inflammatory disorders. Finally, our data show an enrichment of Eomes$^+$IFN-γ$^+$ cells in the synovial fluid of patients affected by juvenile idiopathic arthritis.

P.C1.07.11

Human bone marrow resident natural killer cells have a unique transcriptional profile resembling resident memory CD8$^+$ T cells

***J. E. Melsen**, G. Lugthart, C. Vervat, S. M. Kielbasa, S. A. van der Zeeuw, H. P. Buermans, M. M. van Ostaijen-ten Dam, A. C. Lankester, M. W. Schilham;*
Leiden University Medical Centre, Leiden, Netherlands.

Human lymphoid tissues harbor, in addition to circulating CD56^{bright} and CD56^{dim} natural killer (NK) cells, a third NK cell population: CD69$^+$CXCR6$^+$ lymphoid tissue (lt)NK cells. To obtain more insight in the characteristics of ltNK cells, RNA sequencing was performed on the three NK cell populations from bone marrow and blood. 700-900 genes were differentially expressed between individual populations in blood or marrow. Among the downregulated ltNK cell genes, we identified *S1PR1*, *SELPLG* and *SELL*. By flow cytometry we confirmed that the adhesion molecule (e.g. CD49e$^-$, CD29^{low}, CD81^{high}, CD62L$^-$, CD11c$^-$) and transcription factor profile (e.g. Eomes^{high}, Tbet^{low}) of ltNK cells significantly differed from their circulating counterparts. ltNK cells were characterized by enriched expression of inhibitory receptors TIGIT and CD96, and low expression of DNAM1 and cytolytic molecules (e.g. *GZMB*, *GZMH*, *GNLY*). Their proliferative capacity was reduced compared to the circulating NK cells. Gene set enrichment analysis revealed the transcription factor EGR2 and phosphatase DUSP6 as potential regulators of the ltNK cell transcriptome. Remarkably, comparison of the ltNK cell and published human spleen-resident memory CD8$^+$ T(rm) cell transcriptome revealed a shared transcriptional program. Moreover, the phenotypical profile of ltNK cells resembled that of bone marrow CD8$^+$ Trm cells. Together, we provide molecular data that clearly distinguish ltNK cells from both the circulating CD56^{bright} and CD56^{dim} NK cells and substantiate the view that ltNK cells are tissue-resident and functionally restrained in killing. Our findings underscore the existence of a core gene signature shared between CD8$^+$ Trm and resident NK cells in lymphoid tissues.

P.C1.07.12

Blockade of the T cell ecto-enzyme ARTC2.2 *in vivo* preserves the vitality and function of isolated liver tissue resident-memory T cells

***B. Rissiek**[1], M. Lukowiak[1], F. Raczkowski[2], T. Magnus[1], H. Mittrücker[2], F. Koch-Nolte[2];*
[1]Department of Neurology, Hamburg, Germany, [2]Institute of Immunology, Hamburg, Germany.

On murine T cells, the GPI-anchored ADP-ribosyltransferase 2.2 (ARTC2.2) ADP-ribosylates the P2X7 ion channel at arginine 125 in response to nicotinamide adenine dinucleotide (NAD$^+$) released from damaged cells. We have previously reported that this pathway is activated also by NAD$^+$ released during the preparation of liver leukocytes, where sustained gating of P2X7 by ADP-ribosylation can severely compromise the vitality and function of primary regulatory T cells (Tregs) and NKT cells that co-express high levels of ARTC2.2 and P2X7. In our present study, we evaluated the expression of ARTC2.2 and P2X7 by effector and memory T cells in the liver of mice seven weeks after infection with Listeria monocytogenes (Lm). We found that CD4$^+$ KLRG1$^+$/CD69$^+$ and CD8$^+$KLRG1$^+$/CD69$^+$ tissue-resident memory T cells (Trm) in the liver express high levels of ARTC2.2 and P2X7. Isolation of liver Trm and further incubation at 37°C resulted in cell death of the majority of CD4$^+$ and CD8$^+$ Trm. Injection of the ARTC2.2-blocking nanobody s+16a 30 min prior to organ harvesting prevented ADP-ribosylation of P2X7 during cell preparation and thereby protected Trm from NAD-induced cell death (NICD) when cells were incubated at 37°C. Consequently, preserving Trm vitality by s+16a injection enabled sensitive analyses of the *in vitro* cytokine expression profile of primary liver Trm. In conclusion, *in vivo* blockade of ARTC2.2 during cell preparation by systemically injected nanobody s+16a represents a valuable strategy to study the role and function of liver Trm in mice.

P.C1.07.13

Diagnostic algorithm for the detection of anti-SOX1 antibodies

***R. Ruiz-García**[1], M. García-Ormaechea[1], M. Español-Rego[1], E. Martínez -Hernández[2,3], C. San Bartolomé-Belloch[1], L. Sabater[2], L. Querol[4], I. Illa[4], F. Graus[3,2];*
[1]Immunoloy Department, CDB, Hospital Clínic de Barcelona, Barcelona, Spain, [2]Neuroimmunology Program, Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain, [3]Neurology Department, Hospital Clínic de Barcelona, Barcelona, Spain, [4]Neuromuscular Disorders Unit, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

Introduction: The Lambert-Eaton myasthenic syndrome(LEMS) is a neuromuscular disorder with an associated cancer inup to 50% of patients, mostly lung cancer(LC). The detection of anti-SOX1 antibodies is useful in thediagnosis of paraneoplastic LEMS, although they had been detected sporadically in idiopathic neuropathiesby immunoblot/ ELISA.Currently, there are commercial immunoblots for the detection of anti-SOX1 antibodies but comparisonwith a specific technique is lacking and their sensitivity is unknown. **Methods:** We studied 203 serum samples using cell based assay (CBA), rat cerebellar immunohistochemistry(IHQ) and commercial immunoblot(IB). We included 64 patients with anti-SOX1 antibodies and 139 as diseasecontrols **Results:** 79.7% anti-SOX1 positive patients were male. Mean age was 64 years. 98.4% patients had cancer (61 lung,1 prostate and 1 breast); 25% presented cerebellar degeneration; 22% LEMS; 22% limbic encephalitis; 11%encephalomyelitis; 11% neuropathy/neuronopathy; 3% Stiff-man syndrome; 6% LC without anyneurological syndrome and 2% cerebellar syndrome without cancer. 10.9% anti-SOX1 positive serumsamples by CBA were negative by IHQ but positive by IB (IHQ false negatives) and another 10.9% werenegative by IB (IB false negatives). Regarding the 139 controls, only 1 patient, with sensitive neuropathy andparotid cancer, was positive for SOX1 by IB but seronegative by CBA and by IHQ (IB false positive). Anti-SOX1 abs were detected by all three tests in 78% of patients. **Conclusions:** Anti-SOX1 antibodies are LC markers. It is important to assess the risk of LC in samples negative by IB(>40 years, smoking, classic paraneoplastic syndrome), since 11% of patients have anti-SOX1 antibodies byCBA. False positive results are rare and should be confirmed by CBA if clinical picture is discordant.

P.C1.07.14

Familial multiple sclerosis with autosomal recessive pattern inheritance linked to a region on chromosome 7p

***z. salehi**[1], M. Keramatipour[2], S. Talebi[3], M. Sahraian[4], A. Naser Moghadasi[4], M. Izad[1];*
[1]Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of, [2]Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of, [3]Department of Medical Genetics and Molecular Biology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran, Islamic Republic of, [4]MS Research Center, Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of.

Introduction: The prevalence rate of familial multiple sclerosis (MS) is recently increasing among Iranian population which endorses the heritability of the disease. On the other hand, hereditary disease gene analysis in consanguineous families with multi-incidence of MS could be highly effective in finding mutations underlying disease pathogenesis in an ethnic group. Because of the high rate of consanguinity among Iranian population, we propose to examine genetic mutations in an Iranian family with an autosomal recessive inheritance of relapsing-remitting MS (RRMS).
Materials and Methods: We performed whole exome sequencing (WES) in a family with five patients and four healthy individuals of four generations. Sanger sequencing was done to confirm the found variants. We also performed homozygosity mapping to determine the genomic region linked to the disease.
Results: We described novel homozygous rare variants in *AMPH*, *POLM*, *POLD2* and *TNS3* genes in the affected members. Interestingly, all the variants were located on chromosome 7p arm. Especially, using homozygosity mapping, we found a long continues stretch of homozygosity (LCSH) at chromosome 7p12.3-14.1, spanned more than 10 Mbp, which included four identified variants in the affected proband, his affected father/ sibling.
Conclusion: Our results identify chromosome 7p arm as a genetic region of interest for familial multiple sclerosis susceptibilities which could be considered for the identification disease-causing mutations to solve missing heritability of MS.

P.C1.07.15

A case of autoimmune glial fibrillary acid protein astrocytophaty and a-GFAP autoantibodies

***C. San Bartolomé Belloch**[1], M. Romera Forné[2], M. Antón Monleón[2], L. Ballester-Marco[3], D. Escudero[4], F. Graus[4,5], R. Ruiz-García[2];*
[1]Immunology department CDB, Hospital Clínic de Barcelona., Barcelona, Spain, [2]Immunology department CDB, Hospital Clínic de Barcelona, Barcelona, Spain, [3]Neurology department, Hospital Comarcal de Alcañiz, Teruel, Spain, [4]Neurology department, Hospital Clínic de Barcelona, Barcelona, Spain, [5]Neuroimmunology Program Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain.

INTRODUCTION: Glial fibrillary acidic protein (GFAP) is an intracellular protein located between the smaller microfilaments and larger microtubules in astrocytes and is a target and biomarker for astroglial pathology in neurological diseases. This astrocytopathy is a novel autoimmune central nervous system disorder. The pathogenesis of this disorder is poorly understood and predominantly affects the meninges, brain, spinal cord and optic nerve.
PATIENT AND METHODS: We present a 71 years old patient which showed signs of weakness, tremor, fluctuating episodes of disorientation and bradypsychia. MRI was negative for acute pathology. Lumbar puncture showed presence of leukocytes and elevated protein level. Clinical examination suggested bilateral optic neuritis and lymphocytic meningitis. Serum and cerebrospinal fluid (CSF) samples were tested for antibodies using immunohistochemical assays on rat cerebellum and brain tissues. Antibody specificity was confirmed with an *In house* cell based assay.
RESULTS: Patient's CSF presented a characteristic immunostaining pattern in the rat cerebellum, compatible with a-GFAP antibodies, which were confirmed by indirect immunofluorescence (IFI) with GFAP-transfected HEK293T cells. However, the serum sample did not present any anti-neuronal antibody specificity.
CONCLUSIONS: Here we described a novel case of this rare neurological disease associated with meningoencephalitis and a-GFAP antibodies. We demonstrated, in this case, the intrathecal synthesis of GFAP antibodies by the analysis of both biological samples. It is highly recommended to perform a study as broad and specific as possible in both samples for establish an appropriate diagnosis and treatment.

P.C1.07.16

Apoptosis of food-activated T cells in Peyer´s patches is a hallmark of healthy intestines

U. Steinhoff[1], *S. Hartmann*[1], *K. Rajalingam*[2], *B. Siegmund*[3], *Y. Rodriguez Sillke*[3], *R. Glauben*[3], *A. Visekruna*[1];
[1]*Institute for Microbiology and Hospital Hygiene, Marburg, Germany,* [2]*Institute for Immunology, Mainz, Germany,* [3]*Charité-Medical Department for Gastroenterology, Berlin, Germany.*

It is well known that development of the intestinal immune system is shaped by the microbiota. However little information exists about the impact of dietary antigens on development and homeostasis of the intestine. We studied the impact of dietary proteins on the fate of intestinal immune cells. Data from mice and humans show that continuous exposure to dietary protein antigens leads to highly activated Helios$^+$ Foxp3$^-$ CD4$^+$ T cells in Peyer´s Patches (PP), independently of the intestinal microbiota. Food protein activated, Helios$^+$ CD4$^+$ T cells in PP undergo PD-1 mediated apoptosis and removal of dead cells by macrophages induces local IL-10 production. Further studies in patients with inflammatory bowel diesease revealed significantly reduced frequencies of apoptotic CD4$^+$ T cells as compared to healthy controls. These findings demonstrate that continous activation and subsequent apoptosis of diet-reactive CD4$^+$T cells is a hallmark of the healthy intestine.

P.C1.07.17

Age dependent changes in T cell subsets in multiple sclerosis patients

A. Tejeda Velarde[1], *E. Rodríguez-Martín*[1], *L. Costa-Frossard*[1], *Y. Aladro*[2], *S. Sainz de la Maza*[1], *J. Fernández*[1], *S. Medina*[1], *N. Villarrubia*[1], *E. Monreal*[1], *J. Álvarez-Cermeño*[1], *E. Roldán*[1], *L. Villar*[1];
[1]*Ramón y Cajal University Hospital, Madrid, Spain,* [2]*Getafe University Hospital, Madrid, Spain.*

Introduction: T cells have an important role in multiple sclerosis (MS) physiopathology. CD4 trigger the inflammatory cascade and recruit immune cells into the central nervous system, while CD8 contribute to induce axonal damage. However, age dependent changes in these T cell subsets remain unknown. We aimed to study age dependent changes in CD4 and CD8 subsets in cerebrospinal fluid (CSF) and blood of MS patients.
Patients and methods: We analyzed CSF and blood of 102 MS patients (91 relapsing-remitting and 11 primary progressive) in a FACSCanto II cytometer. We studied: naïve, central memory (CM), effector (Including effector memory, EM, and terminally differentiated, TD), resident memory T cells (T_{RM}), regulatory T cells (Treg), PD-1+ cells and PD-L1+ cells. Results were expressed as percentage. Nonparametric Spearman's correlation was performed to analyze association with age.
Results: In blood, we observed an age dependent decrease in naïve CD8 (r=-0.41; p<0.0001), accompanied by an increase in effector CD8 (r=0.35; p=0.0006), especially in TD CD8 (r=0.33; p=0.0013). We also found an increase in PD1+ CD4 (r=0.48; p=0.0265). These associations were not found in CSF. However, in the CSF we observed an increase in both CD4 (r=0.34; p=0.0017) and CD8 (r=0.25; p=0.0273) $T_{RM.}$ We did not find any association with Treg, but we observed an increase in CD4 PD-L1+ (r=0.56; p=0.0146) in the CSF.
Conclusions: Our data show that the distribution of T cell subsets in CSF and blood changes along the time in MS, and this could have some implications in the prognosis of these patients.

P.C1.07.18

Intra-cerebral T-cells in multiple sclerosis patients recognize EBV-infected cells, but notcandidate disease-associated autoantigens

G. M. G. Verjans*, G. P. van Nierop, R. Q. Hintzen;*
Erasmus MC, Rotterdam, Netherlands.

Introduction. Association between Epstein-Barr virus (EBV) and multiple sclerosis (MS) may involve intracerebral EBV-specific T-cell responses targeting the virus or indirectly, candidate MS-associated autoantigens (cMSAg). We determined the differentiation status and Ag-specificity of T-cells in (i) cerebrospinal fluid (CSF) of early diagnosed MS patients (n=13) and (ii) paired blood, CSF and (non-)affected brain tissue of deceased patients with progressed MS (n=27). **Methods**. T-cells were phenotyped ex-vivo by flowcytometry. Short-term T-cell lines (TCL) were generated from clinical samples by mitogenic stimulation and assayed by intra-cellular cytokine flowcytometry for reactivity towards autologous EBV-infected B-cells (autoBLCL) and transformed autoBLCL expressing eight human cMSAg. **Results**. Intrathecal T-cells in early MS patients are directed to lytic EBV antigens, but not cMSAg. In late stage MS patients, local T-cell responses mainly involved oligoclonal effector-memory cytotoxic CD8 T-cells expressing markers of chronic antigenic stimulation, but not senescence. TCRVß repertoire correlated between paired MS lesions, but with paired unaffected brain tissue, CSF and blood. Whereas no substantial cMSAg-specific T-cell reactivity was detected, brisk CD8 T-cell reactivity was detected in multiple lesion-derived TCL towards autoBLCL. In one MS patient, the autoBLCL-specific T-cell response in paired intra-lesional TCL was dominated by TCRVβ2+CD8+ T cells, which were localized in the parenchyma of the respective tissues expressing a polarized TCR and CD8 expression suggesting immunological synapse formation in-situ. **Conclusions**. The data suggest the involvement of effector-memory cytotoxic CD8 T-cells recognizing antigens expressed by autoBLCL, most likely EBV antigens, but not the assayed human cMSAg in both early and late stage MS patients.

P.C1.07.19

Functional tagging of autoaggressive CD8$^+$ T cells in the animal model of multiple sclerosis

M. Walkenhorst*, A. Willing, E. Luu, N. Kursawe, J. B. Engler, M. A. Friese;*
Institut für Neuroimmunologie und Multiple Sklerose, Zentrum für Molekulare Neurobiologie Hamburg, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.

Multiple sclerosis (MS) is the most common inflammatory disease of the central nervous system (CNS) that is characterised by CNS-infiltrating CD8$^+$ and to a lesser extent also CD4$^+$ T cells. However, the mouse model of MS, experimental autoimmune encephalomyelitis (EAE), is almost entirely driven by autoreactive CD4$^+$ T cells. Therefore, current EAE models only partially reflect the pathogenesis of MS. Accordingly, the role of CD8$^+$ T cells in MS remains elusive and requires a functional characterization of CD8$^+$ T cell responses in EAE that will instruct us on how to genetically engineer a CD8$^+$ T cell-driven model. Here, we genetically and irreversibly labelled activated CD8$^+$ T cells in EAE by using an inducible granzyme B (gzmB) reporter mouse. We found that during EAE onset CD8$^+$ T cells are activated in the periphery and labelled CD8$^+$ T cells were enriched in the CNS compared to draining lymph nodes in the acute phase of the disease. Sequencing the T cell receptor (TCR) of tagged CD8$^+$ T cells in EAE will allow us to identify myelin oligodendrocyte glycoprotein-specific CD8$^+$ T cells. Eventually, we aim to transgenically express identified TCRs to develop a mouse model that enables us to study the pathogenic role of naturally occurring encephalitogenic CD8$^+$ T cells. Therefore, results in this model might allow better translatability to MS.
This work is supported by the Bundesministerium für Bildung und Forschung (BMBF), Krankheitsbezogenes Kompetenznetz Multiple Sklerose (KKNMS).

P.C1.08 Maintenance and local regulation of tissue specific immunity - Part 8

P.C1.08.01

Uterine immune dynamics assessed from pre-pregnancy endometrium to delivery

M. Benner[1], *D. Feyaerts*[1], *C. Cartagena Garcia*[1], *G. Ferwerda*[1], *W. Shadmanfar*[2], *O. W. van der Heijden*[1], *I. Joosten*[1], *R. G. Van der Molen*[1];
[1]*Radboud Institute for Molecular Life Sciences, Nijmegen, Netherlands,* [2]*Mildred Clinics, Arnhem, Netherlands.*

Introduction: The importance of local immunity for correct placental development and healthy pregnancy is evident. Various studies focusing on a particular cell type and time points during pregnancy suggest gestational immune shifts. However, methodological differences between studies make it impossible to fully assess when and to what extent changes in placental immune signature are induced. Here, we examined >30 different lymphocyte (sub)populations and their dynamics during gestation. Methods: Deep immune flowcytometry phenotyping was performed on lymphocytes isolated from the uterine mucosa throughout gestation. Pre-pregnancy endometrial lymphocytes, isolated from menstrual blood, lymphocytes from placenta at different time points (5-11.6wk, 12-14wk and >37wk) and peripheral blood mononuclear cells were processed immediately. Results: NK cells were enriched in endometrium up until the 2nd trimester of pregnancy, while the percentage of T cells in placenta rises after >17wk gestation. Various subpopulations of NK and T cells revealed gestation-dependent shifts. We observed a well-defined B cell population in 1st and 2nd trimester placentae, with highest levels in the 2nd trimester. Especially CD24^{Hi}CD38^{Hi} B cells were increased in 2nd trimester decidua. Currently, we are assessing the nature and putative specificity of these cells. Conclusion: Uterine mucosal immunity changes extensively after the induction of pregnancy. Gestation-dependent local B cell function deserves attention. Contrary to a common believe that decidual B cells are almost absent or only present in pathological conditions, our results highlight possible functional implications in early to mid gestation. Investigation of decidual tolerance-mediating B cells might reveal key mechanisms of how placental immunity is maintained.

P.C1.08.02

A novel human CD3$^+$CD56$^+$regulatory cells: role in the pathogenesis of type 1 diabetes

S. Bruzzaniti[1], *V. Rubino*[2], *C. La Rocca*[1], *A. Giovazzino*[2], *A. Palatucci*[1], *V. De Rosa*[1], *P. de Candia*[3], *S. De Simone*[1], *A. Franzese*[2], *E. Mozzillo*[2], *G. Terrazzano*[4], *G. Ruggiero*[2], *G. Matarese*[2], *M. Galgani*[1];
[1]*Istituto per l'Endocrinologia e l'Oncologia Sperimentale- Consiglio Nazionale delle Ricerche, Naples, Italy,* [2]*University of Naples "Federico II", Naples, Italy,* [3]*Istituto di Ricovero e Cura a Carattere Scientifico MultiMedica, Milan, Italy,* [4]*University of Potenza, Potenza, Italy.*

Regulatory T cells play a cardinal role in the control of immune response and homeostasis. Here, we reported that a circulating T cell population, co-expressing CD3 and CD56 markers (here defined as CD3$^+$CD56$^+$ cells), identifies a novel human regulatory T cell subset, not expressing the fork-head-box-P3 (Foxp3) transcription factor and distinct from NKT cells. Flow-sorted CD3$^+$CD56$^+$ cells showed that CD3$^+$CD56$^+$ cells were able to suppress cytotoxicity and IFN-γ production when co-cultured *in vitro* with CD8$^+$ effector cells. Regulatory function of human CD3$^+$CD56$^+$ cells required cell-to-cell contact and associated to a reduction of intracellular reactive oxygen species (ROS) in CD8$^+$ target cells. Furthermore, through a microarray analysis, we characterized the transcriptional profile of CD3$^+$CD56$^+$ cells, clustering independently from other circulating T cell subset. Finally, these data were corroborated in human autoimmune condition; indeed, peripheral frequency and suppressive function of CD3$^+$CD56$^+$ cells were significantly reduced in a large cohort of children with type 1 diabetes, at diagnosis. Taken together, our findings unveil a previously unrecognized regulatory T cell population specifically controlling CD8$^+$ effector functions, which may play a critical role in the control of immunological tolerance and autoimmune diseases in humans.
JDRF GRANT: 2-SRA-2018-479-S-B
EFSD/JDRF/LILLY European Programme in Type 1 Diabetes Research 2016

P.C1.08.04

Tissue specialization of human Tfr cells

R. Correia[1], *V. R. Fonseca*[1,2], *A. Agua-Doce*[1,3], *L. Graca*[1,3];
[1]*Instituto de Medicina Molecular João Lobo Antunes, Lisboa, Portugal,* [2]*Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Lisboa, Portugal,* [3]*Instituto Gulbenkian de Ciência, Oeiras, Portugal.*

The recent discovery of a follicular regulatory T (Treg) cell subset, called T follicular regulatory (Tfr) cell, has revealed a new and specialized mechanism for germinal centre (GC) regulation and prevention of autoimmunity. Tfr cells gain access to the GC and suppress follicular helper T (Tfh) cells and B-cell responses. In humans, blood Tfr cells are not fully competent in suppressing humoral responses. However, full knowledge of human Tfr cell biology have been challenged by the difficulty to study secondary lymphoid organs.
We studied Tfr cells in human lymph nodes and tonsils. We found that the presence of GC reactions impacts the phenotype of Tfr cells. While in human lymph nodes without GC reactions (identified by Bcl-6 immunohistochemistry), Tfr cells resemble their blood counterparts, GC presence is associated with the emergence of activated Tfr cells, as the ones usually found on human tonsils.
Taken together, these results suggest that human Tfr cells follow a tissue compartment specialization accordingly to the presence of ongoing humoral responses. A detailed phenotypical and functional analysis of Tfr cells in different human tissues is critical to understand the biology of these cells.

P.C1.08.05

Potential role of CD4+T cell-derived extracellular microRNAs in the loss of immune tolerance during multiple sclerosis

S. Garavelli[1], *F. Buttari*[2], *F. Carbone*[3], *C. Procaccini*[3], *V. De Rosa*[3], *D. Centonze*[2], *G. Matarese*[3], ***P. de Candia***[1];
[1]*IRCCS Multimedica, Milan, Italy,* [2]*IRCCS Istituto Neurologico Mediterraneo (INM) Neuromed, Pozzilli, Italy,* [3]*Istituto di Endocrinologia e Oncologia Sperimentale, Consiglio Nazionale delle Ricerche (IEOS-CNR), Naples, Italy.*

Introduction: CD4$^+$ T regulatory (Treg) cells control inflammation by inhibiting the growth and cytokine production of CD4$^+$ T conventional (Tconv) cells. We showed that, upon stimulation, T cell subsets release extracellular vesicles (EVs), containing distinct patterns of microRNAs with the capability of modulating specific mRNA targets upon cellular up-take. Objective of the present work was to evaluate whether the T cell-derived extracellular microRNA signatures may get dysregulated in autoimmunity with a potentially different impact on the transcriptome of EV receiving cells. Materials and Methods: To this aim, human CD4$^+$ T cell subsets were purified from peripheral blood of naïve to treatment relapsing-remitting multiple sclerosis (RRMS) patients and healthy controls. Tconv- and Treg cells were *in vitro* stimulated and released EVs were characterized by nanoparticle tracking analysis and RT-qPCR to analyze size distribution and microRNA content. Results: Compared to healthy, both Tconv and Treg cells from patients showed a differentially expressed set of microRNAs with crucial regulatory function in the immune system. By a mRNA sequencing approach, we have then demonstrated that the treatment of naïve T cells with Treg- (but not Tconv-) EVs caused the specific repression of genes involved in the proteasome-dependent proteolytic process, known to be crucial for T cell activation, and that in RRMS, Treg-derived EVs may have lost this capability. Conclusions: Our results unveil a novel molecular mechanism for Treg-mediated maintenance of self-tolerance and its potential alteration in multiple sclerosis. Grants: National Multiple Sclerosis Society NMSS (PP-1606-24687) and Fondazione Italiana Sclerosi Multipla FISM (2016/R/10) to PdC.

P.C1.08.06

The role of regulatory T cells in the development of obesity in MIF-KO mice

D. Gajić, *I. Koprivica, M. Vujičić, I. Stojanović, T. Saksida;*
Institute for Biological Research "Siniša Stanković", Belgrade, Serbia.

Obesity is a disorder characterized by a pro-inflammatory environment in visceral adipose tissue (VAT) due to increased infiltration of pro-inflammatory macrophages and a drop in regulatory T (Treg) cells. Macrophage migration Inhibitory Factor (MIF) is a pro-inflammatory cytokine with versatile functions in innate and adaptive immunity. Although it has a predominantly pro-inflammatory role in the organism, its innate absence in MIF-KO mice leads to obesity. VAT in MIF-KO mice is larger in mass and the infiltration of immune cells per gram of VAT is not different than in WT controls. Also, MIF-KO VAT has the same distribution of immune cells (CD3+, CD4+, CD8+, CD19+ cells, M1 and M2 macrophages), but a higher expression and secretion of TNF-α and IL-1β. Surprisingly, Treg cells are more abundant in VAT of MIF-KO mice. Proliferation of Treg cells in VAT measured by BrdU incorporation is the same in both strains, suggesting that their increased number is not due to enhanced *in situ* division. Cytokines responsible for Treg suppressive action, IL-10 (denoted as CD4+IL-10+FoxP3+ cells) and TGF-β (secreted from VAT infiltrating cells), are underrepresented in VAT of MIF-KO mice. Based on these results, we can assume that Treg cells in VAT of MIF-KO mice are, albeit extensively present, less functional. This situation may be responsible for obesity development in the absence of MIF. *Supported by Ministry of Education, Science and Technological Development, Republic of Serbia (#173013)*

P.C1.08.07

Exploratory study on the association of Vγ9Vδ2Tcells with Chlamydial cervico-vaginal infection in women with Recurrent Spontaneous Abortions

I. Voskakis[1], *C. Tsekoura*[1], *T. Keramitsoglou*[1], *E. Deligeoroglou*[2], ***I. Gkougkourelas***[3], *E. Glynou*[4], *M. Varla-Leftherioti*[1];
[1]*Dept. of Immunology and Histocompatibility "Helena Venizelou" Hospital, Athens, Greece,* [2]*National and Kapodistrian University of Athens. School of Medicine. Aretaieio Hospital, Athens, Greece,* [3]*2nd Dept. of Internal Medicine, Aristotle University of Thessaloniki, Hippokratio GHTH, Thessaloniki, Greece,* [4]*Dept. of Microbiology "Helena Venizelou" Hospital, Athens, Greece.*

Introduction: We have previously reported a significant association of Vγ9Vδ2T cells with cervico/vaginal *Chlamydia trachomatis*(*Ct)*infection in women with RSA. This association raises questions on the chlamydial antigens that activate γ9δ2 T cells to develop an anti-trophoblast response as well as the factors that enhance it. In the present study, we try to answer if the γ9δ2-mediated anti-trophoblast activity is related to the length of the infection by analyzing the γ9δ2T cells in aborters who have received or not anti-clamydial treatment.**Patients-Methods:** The percentage of γ9δ2T cells in PB was analyzed by flow cytometry in 76 positive for *Ct* infections (*Ct*+) RSA women (A), within 10 days after a new miscarriage. 35 of them had received anti-*Ct* treatment upon diagnosis (T) and 41 were not treated (NT). Fertile women without Ct infection (*C*) served as controls(C=82). **Results:** A highly statistically significant difference of mean percentages of γ9δ2T cells was shown between Tand C groups (58.95 vs 61.92, p<0.00001). The analysis performed in T and NT aborters revealed that the mean percentage was 58.95% and 83.29% respectively (p=0.0002) and that 53% of NT women had >80% γ9δ2T cells (only 20% of T women, p=0.0017). **Conclusions:** The increased levels of PB γ9δ2T cells in untreated cases support the hypothesis that the persistence of *Ct* in the cervic/vaginal tract favors the recognition by γ9δ2T cells *Ct* antigens sharing common epitopes with antigens (HSP?) expressed on the embryonic tissues which results in the activation of anti-embryonic responses and possibly abortions.

P.C1.08.08

Sertoli cells have non-canonical functional inflammasome network able to perturb testis niche immune tolerance and to inflict cell death

K. Todorova, E. Avramska, L. Sezer, A. Apostolova, ***S. Hayrabedyan****;*
Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria.

Sertoli cells are pivotal in protecting auto-immunogenic germ cells by establishing an immune privileged environment, but eventually they could also serve as germline damage sentinels. We have recently showed a functional Nrp3 inflammasome in mouse Sertoli cells, both primary and cell line, where challenging of NOD1/NOD2 and TLR4, in ATP presence activated caspase-1, incurring cell death and mature IL-1β, IL-6, IL-23 secretion.
We explored if the caspase-1 mediated cell death was pyroptosis-like and it was only Nlrp3 regulated.

Surprisingly, we found an even larger non-canonical innate immunity inflammasome network elicited by TLR4/NOD1/ATP challenge (NGS RNA-Seq: intact Sertoli vs. Ma, Illumina; challenged vs. intact Sertoli cells, Oxford Nanopore), that was preserved in metabolic stress (caspase-1, RT-qPCR) and consisted of several inflammasomes and adaptors. Caspase-1 inflammasomes Aim2 (9000 fold), Nlr10 (7000 fold), Nlrp3 (200 fold) and Nlrc4 (100 fold) upregulation overwhelmed anti-apoptotic Naip1 (100 fold). The importance of metabolic preference for glycolysis (Agilent Seahorse) was supported by the role of MAPK1 for NF-κB upregulation upon challenge (MAPK1 siRNA in stable 15P-1 cell line with pNIFTY2-SEAP reporter). Sertoli cells undergo partial pyroptosis-like cell death (PI imaging, flow cytometry), with membrane leakage (LDH assay), blebbing (live imaging, fluorescent reporter), but without pyroptosis-like membrane shredding.
Inflammasome network serves as sentinel for germline genome stability by sensing DAMP/PAMP/metabolic stress, acting through caspase-1 axis, a notion coinciding with clinically observed semen IL-1β/IL-18 finding in male infertility patients, suggesting new therapy avenues.
Acknowledgement: Bulgarian National Science Fund - DKOST 01/23, 2017; Bulgarian Academy of Sciences - DFNP 17/141-2017

P.C1.08.09
The alarmin IL-33 drives a ST2$^+$ Treg-mediated anti-inflammatory immune response during immune-mediated hepatitis

***F. Heinrich**, A. Ochel, G. Tiegs, K. Neumann;*
University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Introduction: During Concanavalin A (ConA)-induced immune-mediated hepatitis, the alarmin IL-33 is released by necrotic hepatocytes and induces immune responses by signaling through the IL-33 receptor ST2. We have shown previously that IL-33 pre-treatment prevents development of immune-mediated hepatitis suggesting an immunosuppressive function for IL-33 in liver disease. Since regulatory T cells (Tregs) respond to IL-33, we aimed to investigate the IL-33-driven Treg response in the inflamed liver.
Methods: C57BL/6 mice were treated either with IL-33 or PBS alone or received ConA after 3-day IL-33/PBS treatment. Mice were analyzed 24 hours after hepatitis induction. The phenotype of Foxp3$^+$ Tregs was determined by flow cytometry.
Results: We showed that the frequency of hepatic Foxp3$^+$ Tregs was significantly increased in IL-33-treated mice compared to PBS-treated mice. Within the Treg population, the frequency of Tregs expressing ST2 was highly elevated. Compared to ST2$^-$ Tregs, hepatic ST2$^+$ Tregs displayed an activated phenotype with up-regulated expression of CD25, KLRG1, and ICOS as well as enhanced expression of the functional markers CTLA-4, TIGIT, and GITR. During immune-mediated hepatitis, the frequency of hepatic ST2$^+$ Tregs was elevated compared to healthy mice and was further strongly increased by IL-33 pre-treatment. Interestingly, hepatic ST2$^+$ Tregs also have an activated phenotype in liver inflammation that was not altered by IL-33 pre-treatment.
Conclusion: The immunosuppressive function of IL-33 in immune-mediated hepatitis might be driven by expansion and recruitment of a highly activated Treg population expressing ST2.

P.C1.08.10
Dendritic cells as predictors for embryo implantation

***C. Kyvelidou**, A. Geisler, B. Toth, C. Heufler, S. Hofer-Tollinger;*
Medical University of Innsbruck, Innsbruck, Austria.

The immunological processes surrounding implantation and pregnancy are still under investigation. Promising players are dendritic cells (DC), major controllers of the immune system and key immune cells in the human endometrium. We hypothesize that the mechanisms for the establishment of maternal tolerance are generated even before implantation, are highly depended on DC for their initial activation, and that embryo-produced bioactive factors trigger DC for tolerance. Discarded medium (SM) from human single-embryo cultures was collected in order to perform a large scale protein array and also to be used for the treatment of DC. Our goal was to compare the outcomes between medium from embryos that resulted in pregnancy (prSM) and the ones that did not (npSM). Preliminary results so far show a different protein expression pattern in the SM which can be associated with a positive or a negative pregnancy outcome. Furthermore, several DC genes were found to be regulated by the SM. VCAM-1, CXCL2, CCL19, CXCL10, CXCL8, RELB, and NFKB1 were found upregulated by the prSM, while CCL2 was found upregulated by the npSM. These results indicate that indeed preimplantation embryos are able to produce bioactive factors that can affect DC. The elevated number of DC during pregnancy and their ability to interact with T regulatory and natural killer cells render DC a very promising target in the field of maternal tolerance and their study can offer new insights in the immunological events surrounding implantation and early pregnancy and inspire novel immunomodulatory strategies and treatment options for infertility.

P.C1.08.11
Myeloid-specific molecular mediators of subchondral bone damage in antigen-induced arthritis

***N. Lukač**[1,2], M. Fadljevic[1], I. Radanovic[1], E. Lazic Mosler[3,4], A. Sucur[1,5], D. Flegar[1,5], T. Kelava[1,5], V. Katavic[1,2], D. Grcevic[1,5], N. Kovacic[1,2];*
[1]Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia, [2]Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia, [3]General Hospital Dr. Ivo Pedišić, Sisak, Croatia, [4]Catholic University of Croatia, Zagreb, Croatia, [5]Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia.

Introduction: Rheumatoid arthritis (RA) is marked by subchondral bone destruction, joint deformations and disability. Available therapeutics improve the prognosis, but with limited effect on bone destruction. Using antigen-induced arthritis (AIA), animal model of RA, we found that mice deficient for Fas gene (Fas-/-) develop non-destructive arthritis, marked by lower frequency of myeloid cells in joints. We aim to identify mediators of bone resorption in arthritis, by analyzing differentially expressed genes in sorted myeloid population from wild-type (WT) and Fas -/- mice with AIA.
Materials and methods: AIA was induced by intra-articular injection of methylated bovine serum albumin to immunized mice. Bone resorption was assessed by µCT. Synovial cells were released by collagenase, labeled with anti-mouse CD45-FITC, CD11b-PE, Gr1-PECy7, B220/CD3/NK1.1/CD31/TER119-APC, and CD51-APCeF780. CD11b+Gr-1+ population was sorted using BD FACSAria, RNA extracted by Trizol, and hybridized to Affymetrix ST 2.0 arrays. Differences in gene expression found on arrays were confirmed by qRT-PCR.
Results: Synovial CD11b+Gr1+ cells were transcriptionally similar in Fas -/- and WT mice with AIA. Samples split into two hierarchical clusters consisting predominantly of Fas -/- or WT samples. WT-dominant cluster revealed up-regulated genes related to cell cycle progression and mitosis, suggesting higher proliferation. Mid1 and Erdr1 genes were down-regulated in joints from Fas -/- mice with AIA, which was confirmed by PCR.
Conclusions: Resorptive AIA is marked by higher myeloid proliferation potential. Mid1 gene is a potential novel mediator for targeting inflammation-mediated joint destruction in arthritis.
Grants: This work was supported by the Croatian Science Foundation project number 7406.

P.C1.08.12
Functional analysis of inflammation associated parameters in a patient with NEMO deficiency

***N. Sürücü**[1], B. Kayaoglu[1], E. Alpdündar Bulut[1], I. C. Ayanoglu[1], E. Dünüroglu[1], M. Acar[1], B. Sözeri[2], A. Kıykım[3], E. Karakoç-Aydıner[3], S. Barıs[4], A. Özen[3], M. Gürsel[1];*
[1]Middle East Technical University, Department of Biological Sciences, Ankara, Turkey, [2]Health Sciences University Ümraniye Teaching and Research Hospital, Pediatric Rheumatology Clinic, İstanbul, Turkey, [3]Marmara University Hospital, Pediatric Allergy and Immunology Department, İstanbul, Turkey, [4]Marmara University Hospital, Pediatric Allergy and Immunology Department, Istanbul, Turkey.

NEMO (NF-κB essential modulator, IKK-γ), is a regulatory component of the IKK (inhibitor of NF-κB kinase) complex and has a central role in the activation and subsequent translocation of the nuclear factor (NF)-κB transcription factor. NEMO deficiency, is a rare type of primary immune deficiency which has also been associated with autoinflammatory manifestations. This study aimed to investigate underlying mechanisms of inflammation in a patient with NEMO deficiency that presented with recurrent fever, perivascular and interstitial neutrophilic infiltrates and nodular skin lesions. PBMC and neutrophils were isolated from peripheral blood and used for functional assays and gene expression analysis using the NanoString inflammation panel. PBMC of the patient and healthy controls were stimulated with various TLR ligands or anti-CD3/anti-CD28. Results showed decreased cytokine responses in the patient (IL-1β, IL-6, IL-17, IFNγ) compared to healthy individuals. However, type I IFN responses to cytosolic nucleic acids was uncompromised. Low density granulocytes (LDG) in the PBMC fraction were abnormally increased in the patient and gene expression analysis subsequently confirmed a striking increase in MMP9 expression. NanoString pathway analysis showed a decreased score for IKK and NF-κB signaling pathway but an upregulation of type I interferon related genes MX1 and IFI44 and an elevated JAK/STAT pathway score. Consistently, cytometric bead array (CBA) analysis of plasma showed a sixty-fold increase in circulating IP-10 levels in the patient compared to healthy controls. Collectively, these results suggest that inflammation-associated symptoms in the patient might stem from elevated type I interferons and LDGs, leading to granulocyte dysregulation.

P.C1.08.13

Hypoxia and rheumatoid phenotype decrease the T helper cell suppressive capacity of synovial fibroblasts

*N. Kaul[1], S. R. Mohapatra[2], T. Tretter[1], C. A. Opitz[2], H. Lorenz[1], **L. Tykocinski**[1];*
[1]University of Heidelberg, Heidelberg, Germany, [2]German Cancer Research Center (DKFZ), Heidelberg, Germany.

Introduction: The pathogenesis of rheumatoid arthritis (RA) is linked to functional changes in synovial fibroblasts (SF) and local infiltration of T lymphocytes. Increased synovial inflammation is also associated with a hypoxic microenvironment. Oxygen levels in the joints of RA patients are significantly decreased (mean oxygen tension: 3.2%) compared to those of osteoarthritis (OA) patients. So far, little is known about the effects of hypoxia on the interaction between fibroblasts and T cells and its implications on the pathophysiology of RA. Methods: SF of OA or RA patients were co-cultured with Th cells under normoxic or hypoxic (3% O_2) conditions. Th cell proliferation was determined by flow cytometry, cytokine secretion by ELISA, indoleamine 2,3-dioxygenase 1 (IDO1) expression was analysed by Western Blot and real-time PCR, tryptophan/ kynurenine levels were quantified by HPLC. Results: Under normoxic conditions, SF strongly inhibited the proliferation of co-cultured Th cells by an IDO1-mediated depletion of tryptophan. RASF showed a significantly lower IDO1 expression, tryptophan metabolism and a significantly weaker capacity to suppress the proliferation of Th cells compared to OASF. Under hypoxic conditions, the expression of IDO1, the tryptophan metabolism and the Th cell suppressive capacity of both OASF and RASF were significantly reduced. Conclusions: The IDO1-mediated suppression of Th cell growth may play an important role in preventing inappropriate Th cell responses under normal conditions. The reduced tryptophan metabolism under hypoxia together with the inferior efficiency of RASF to restrict T cell proliferation likely supports the development of synovitis in RA.

P.C1.08.14

Increase of aerobic glycolysis mediated by activated T helper cells drives synovial fibroblasts towards an inflammatory phenotype

*P. Kvacskay[1], M. M. Souto-Carneiro[1], R. Carvalho[2], K. D. Klika[3], H. Lorenz[1], **L. Tykocinski**[1];*
[1]University of Heidelberg, Heidelberg, Germany, [2]University of Coimbra, Coimbra, Portugal, [3]German Cancer Research Center (DKFZ), Heidelberg, Germany.

Introduction: There is growing evidence for a dysregulated glucose metabolism of synovial fibroblasts (SF) in rheumatoid arthritis (RA) being a prerequisite for their aggressive phenotype. As yet, little is known about the influence of immune cells on the metabolism of SF although local infiltration of leucocytes constitutes a hallmark in the pathogenesis of RA. Here, we investigated the effect of Th cells on the glucose metabolism and cytokine production of non-inflammatory (osteoarthritis (OA)) and inflammatory (RA) SF. Methods: RASF and OASF were cultured in the presence of a stable glucose isotope ($U^{-13}C$) and stimulated with culture supernatants (SN) of activated Th cells. Glucose and lactate levels were determined by proton nuclear magnetic resonance spectroscopy (H-NMR). Cytokine secretion was quantified by ELISA. Janus kinases (JAKs) were blocked by Baricitinib or Tofacitinib, glycolysis was inhibited by 3-Bromopyruvate or Fx11. Results: Resting RASF showed a significantly higher production of lactate, IL-6 and MMP-3 compared to OASF. Stimulation by activated Th cells strikingly changed the metabolic profile of both SF by inducing a shift towards aerobic glycolysis with strongly increased lactate production. In parallel, a significant increase in IL-6 and MMP-3 secretion was induced. Interestingly, blocking of either JAKs or of glycolysis significantly reduced both the production of lactate and the secretion of inflammatory cytokines. Conclusions: A Th cell-mediated metabolic switch towards aerobic glycolysis in SF could likely be an important step in the pathogenesis of RA. Targeting this mechanism by JAK- or glycolytic inhibitors may provide a new strategy in RA therapy.

P.C1.08.15

Mammary gland involution stimulates severe local inflammation that promotes breast cancer tumourigenesis

***A. Unsworth**[1], R. Anderson[2], N. Haynes[3], K. Britt[3];*
[1]Peter MacCallum Cancer Centre, Department of Oncology, Melbourne, Australia, [2]Olivia Newton-John Cancer Wellness & Research Centre, Heidelberg, Australia, [3]Sir Peter MacCallum Cancer Centre, Department of Oncology, Melbourne, Australia.

During reproduction the breast undergoes significant structural changes to prepare for breastfeeding. Once breastfeeding ceases, the mammary gland undergoes rapid tissue remodeling, which is likened to a wound-healing response. While this process is tightly controlled, the wound-healing environment during involution is also known to be ideal for tumor development. Preliminary transcriptional analysis suggests the immune system may play a critical role in increasing breast cancer risk during involution, as inflammatory markers are significantly up-regulated in the involuting mammary gland.
Our study aims to examine the specific composition of immune cells in the mouse mammary gland during reproduction, specifically involution (repair) and parous (resting) using a multi-panel flow cytometry approach and cytokine bead analysis. To determine the effects of these reproductive environments on breast tumor development, syngeneic mammary tumor cells were introduced orthotopically into involuting, parous and nulliparous mice.
The involuting mammary gland exhibits a significant increase in myeloid cells, coupled with decreases in T and B-lymphocytes and expansion of pro-inflammatory cytokines compared to nulliparous glands. This inflammatory environment stimulates mammary tumor growth at a faster rate compared to that of a resting nulliparous gland. The parous mammary gland (assessed 9-weeks post-involution) showed complete resolution of this inflammatory microenvironment, consequently having no effect on tumor growth rate in mice.
The involuting mammary gland exhibits a strong inflammatory microenvironment, which we show promotes the development of breast cancer. We are currently investigating immune targeted therapeutics that could be used to reduce this local inflammation and consequently decrease breast cancer risk during involution.

P.C1.08.16

Human myometrial CD4 T cells at the maternal-fetal interface are tissue-resident memory T cells, which show site-specific adaptation within the uterus

***J. Wienke**[1], L. Brouwers[1], T. E. Vogelvang[2], A. Franx[1], B. B. Van Rijn[1], F. Van Wijk[1];*
[1]UMC Utrecht, Utrecht, Netherlands, [2]Diakonessenhuis, Utrecht, Netherlands.

Introduction: The uterine myometrium is a unique immune environment, capable of harboring a 'foreign' fetus without eliciting an immune response. We aimed to investigate the presence, adaptation and function of human myometrial CD4 T cells at the maternal-fetal interface in uncomplicated pregnancy.
Methods: Myometrial biopsies were obtained at caesarean section from placental and incision site. Lymphocytes were isolated through dissection and digestion with collagenase IV. CD4 T cells were analyzed by flow cytometry or FACS sorted for RNA sequencing by CEL-seq protocol. Suppression assays were performed with FACS sorted uterine regulatory T cells (Tregs).
Results: 70-80% of myometrial CD4 T cells were CD69+, suggesting a tissue-resident memory (TRM) phenotype. RNA sequencing confirmed a TRM-like profile in CD69+ cells, with high expression of CD49a, CXCR6, DUSP6, PD-1 and low expression of CD62L, KLF2/3 and S1PR1 compared to blood memory CD4 T cells. Interestingly, expression of negative costimulatory molecules such as PD-1, TIGIT, Lag3, TIM-3 and CTLA4 on the CD4 T cells, as well as the percentage of Treg cells, was higher at the placental site compared to the incision site of the uterus. Suppression assays confirmed the suppressive capacity of myometrial Tregs.
Conclusion: Myometrial CD4 T cells at the maternal-fetal interface are TRM cells with a high expression of negative costimulatory molecules and a high abundance of functional Tregs. The topographical distribution of these immunoregulatory features indicates that T cells may not only adapt to a tissue, but also to specific sites within one tissue, possibly depending on the local micro-environment.

P.C1.08.17

Defective Regulatory T cells and B cell Subsets Are Associated with Autoimmunity in Common Variable Immunodeficiency Patients

***R. Yazdani**, G. Azizi, H. Abolhassani, F. Kiaee, A. Mirshafiey, A. Aghamohammadi;*
Research Center for Immunodeficiencies (RCID), Tehran, Iran, Islamic Republic of.

Introduction: Common variable immunodeficiency (CVID) is one of the most prevalent symptomatic primary immunodeficiencies (PIDs), which manifests a wide clinical variability such as autoimmunity, as well as T cell and B cell abnormalities. **Methods:** A total of 72 patients with CVID were enrolled in this study. Patients were evaluated for clinical manifestations and classified according to the presence or absence of autoimmune disease. We measured regulatory T cells (Tregs) and B-cell subsets using flow cytometry, as well as specific antibody response (SAR) to pneumococcal vaccine, autoantibodies and anti-IgA in patients. **Results:** Twenty-nine patients (40.3%) have shown at least one autoimmune manifestation. Autoimmune cytopenias and autoimmune gastrointestinal diseases were the most common. A significant association was detected between autoimmunity and presence of hepatomegaly and splenomegaly. Among CVID patients, 38.5% and 79.3% presented a defect in Tregs and switched memory B-cells, respectively, whereas 69.0% presented CD21low B cell expansion. Among patients with a defect in Treg, switched memory and CD21low B cell, the frequency of autoimmunity was 80.0%, 52.2% and 55.0%, respectively. A negative correlation was observed between the frequency of Tregs and CD21low B cell population. 82.2% of patients had a defective SAR which was associated with the lack of autoantibodies. **Conclusion:** Autoimmunity may be the first clinical manifestation of CVID, thus routine screening of immunoglobulins is suggested for patients with autoimmunity. Lack of SAR in CVID is associated with the lack of specific autoantibodies in patients with autoimmunity. It is suggested that physicians use alternative diagnostic procedures.

P.C1.08.18

Regulatory function of B cells on T cell immunity during viral infection is controlled by the surface receptor Toso

***J. Yu**, N. Föger, K. Lee;*
Inflammation Research Group, Institute of Clinical Chemistry, Hannover Medical School, Hannover, Germany.

The immune system is tightly controlled by regulatory processes that allow for the elimination of invading pathogens, while limiting immunopathological damage to the host. In this study, utilizing conditional gene deletion, we demonstrate a critical immunoregulatory role of the cell surface receptor Toso, that, via a B cell-inherent mechanism, provides protective T cell immunity against viral infection. Employing conditional gene deletion, our study revealed that impaired anti-viral T cell responses in Toso deficient mice, were not due to T cell inherent defects, but rather induced by a previously unrecognized function of Toso in B cells. We specifically demonstrated that the deletion of Toso in B cells results in impaired inflammatory T cell responses, such as production of TNFα and IFNγ, in response to influenza A infection. Further studies showed that Toso deficiency in B cells results in a strong increase of IL-10-competent B cells *in vivo*, and, as we further demonstrate through adoptive transfer experiments, this specific subtype of B cells mediates immunosuppressive activity on T cell immunity during influenza A infection. Thus, Toso exhibits its immunoregulatory function by controlling a pool of IL-10-competent regulatory B cells. In addition, we demonstrate that during influenza A-induced pulmonary inflammation the application of Toso-specific antibodies selectively induces IL-10-competent B cells at the site of inflammation and results in decreased proinflammatory cytokine production by lung T cells. These findings suggest that Toso may serve as a novel therapeutic target to dampen pathogenic T cell responses via the modulation of IL-10-competent regulatory B cells.

P.C1.08.19

Roquin deficiency in T cells induces early stages of pancreatic cancer

***T. Raj**[1], J. Zöller[2], D. Hu[1], G. Bianco[2], M. Heikenwälder[2], V. Heissmeyer[1,3];*
[1]Biomedical Center Munich, Institute for Immunology, LMU Munich, Planegg - Martinsried, Germany, [2]Department of Chronic Inflammation and Cancer, German Cancer Research Center, Heidelberg, Germany, [3]Research Unit Molecular Immune Regulation, Helmholtz Zentrum München, Munich, Germany.

Our immune system not only prevents cancer development by eliminating transformed cells, it can also drive chronic inflammation causing tissue insult and the development of cancer. Acute and chronic pancreatitis are strongly implicated in the development of pancreatic ductal adenocarcinoma (PDAC), the most frequent form of pancreatic cancer and one of the leading causes of cancer deaths worldwide.
Investigating mice with combined Roquin-1 and Roquin-2-deficiency in T cells we show that chronic T cell activation can lead to autoimmune pancreatitis, acinar-to-duct metaplasia (ADM) and formation of PanIN1A (pancreatic intraepithelial neoplasia) lesions in mice. Pancreatic damage increases with age, with half of the mice aged 10 - 20 weeks exhibiting precursor lesions. Pancreata were infiltrated with inflammatory cells and pSTAT3 levels were strongly increased, pointing towards a potential role of IL-6 mRNA deregulation in T cells during the development of Roquin mediated pancreatic pathology. These mice produced autoantibodies against pancreatic antigens, suggesting an involvement of Tfh cells. Combined genetic inactivation of the Roquin encoding alleles as well as of its target Ox40 lead to a partial rescue and an improved phenotype. Currently, we are investigating the contributions of Tfh and Th17 cells to the pancreatic pathology. Furthermore, we are evaluating antibodies against Roquin dependent surface antigens as potential biomarkers for PDAC in mice and humans.
We propose that mice with conditional Roquin deletion in T cells are a useful model to study how chronic inflammation and autoimmune pancreatitis trigger the development of neoplasia and pancreatic cancer.

P.C1.08.20

Expression Pattern of TIPE1 Insights into its Functions

***S. Liu**, J. Shao, Y. Li, G. Jin, C. Gao;*
Department of Immunology, Ji'nan, China.

Members of the tumor necrosis factor-alpha-induced protein-8 (TNFAIP8 or TIPE) family play important roles in immune homeostasis and cancer. TIPE1 (TNFAIP8-like 1) is a new member of the TIPE family that may regulate cell death. We found that TIPE1 protein was detected in a wide variety of tissues in C57BL/6 mice and a variety of cells of the epithelial origin, particularly those with secretory functions. High levels of TIPE1 mRNA were detected in most human carcinoma cell lines. TIPE1 is also detectable in endothelial cells, which can induce endothelial dysfunction when exposed to oxidative stress and resulting in atherogenesis in ApoE-/- mice.

P.C1.08.21

Characteristics of gamma/delta T cells at the feto-maternal interface of murine pregnancy

***J. M. Nörenberg**[1], M. Meggyes[1,2], P. Jáksó[3], A. Barakonyi[1,2];*
[1]Department of Medical Microbiology and Immunology, Medical School, University of Pecs, Pécs, Hungary, [2]János Szentágothai Research Centre, Pécs, Hungary, [3]Department of Pathology, Medical School, University of Pécs, Pécs, Hungary.

Pregnancy is an immunological enigma where paternal antigens are present at the feto-maternal-interface. What regulates the local immunotolerance which is necessary to prevent rejection of the conceptus is still under strong investigation. Gamma/delta T cells are believed to play a role in the local regulation of this immunotolerance towards the semi-allogenic fetus. Gamma/delta T cells from uterus and spleen of pregnant and non-pregnant mice were analyzed by flow cytometry.The ratio of γδT cells in the decidua associated lymphoid tissue increases during the course of murine pregnancy. Those decidual γδT cells are in large part $\gamma\delta TCR^{dim}/CD4^{+}$, whereas $\gamma\delta TCR^{bright}$cells are mainly $CD4^{-}$. Furthermore, compared to peripheral γδT cells, a greater proportion of decidual γδT cells express CD107a, TIM-3 and TIM-1, by contrast no difference in the expression of CD160 was detected. Within lymphocytes expressing CD107a, TIM1 or CD160, the rate of γδT cells is significantly higher in the decidua. Accordingly, γδT cells seem to influence the Th1-Th2 balance, which is crucial to provide protection against pathogens, while mediating tolerance towards the semi-allograft.This study was supported by a grant from the University of Pécs (AOK-KA/13-03/34039) and by TÁMOP 4.2.4. A/2-11-1-2012-0001. The present scientific contribution is dedicated to the 650th anniversary of the foundation of the University of Pécs, Hungary.

P.C2.01 Immune signaling and therapy in autoimmunity - Part 1

P.C2.01.01

Identification and characterization of novel toll-like receptor 3 mutations in patients with autoimmune Addison's disease

***S. Aslaksen**, E. Bratland, E. Husebye;*
Department of Clinical Science and KG Jebsen Center for autoimmune diseases, Bergen, Norway.

Autoimmune Addison's disease (AAD) is a classic organ-specific autoimmune disease characterized by an immune-mediated attack on the adrenal cortex. As most complex autoimmune diseases, AAD is believed to be caused by a combination of genetic and environmental factors. Prolonged or persistent viral infections have been suggested to play an important role in triggering the autoimmune destruction, by invoking increased inflammation and autoimmunity. Such infections can be caused by aberrations in innate immunity, including mutations in Toll-like receptor 3 (TLR3). TLR3 recognizes double stranded RNAs, and is therefore a major factor in antiviral defense. It is expressed and functional in the adrenal cortex, which has been suggested to serve as a reservoir of infection for several viral species. Here we report two novel missense mutations, F351I and G221R, as well as three previously described mutations, T59N, R867Q and L412F, in TLR3 in patients with AAD. Their biological implications on TLR3's function were evaluated in cell-culture models, revealing a devastating effect of F351I and R867Q. F351I was present in two Norwegian patients with AAD, but was not found in 379 Norwegian blood donors or in the Exome Aggregation Consortium (ExAC) database. Together, these findings suggest a potential role for TLR3 in the pathogenesis of AAD.

P.C2.01.02

The anti-inflammatory neuropeptide cortistatin plays a critical role in the development and progression of atherosclerosis

***R. Benítez**[1], I. Forte-Lago[1], F. O'Valle[2], M. Delgado[1];*
[1]Institute of Parasitology and Biomedicine Lopez Neyra, CSIC, Granada, Spain, [2]Department of Pathology, School of Medicine, University of Granada, Granada, Spain.

Atherosclerosis is a chronic inflammatory and autoimmune cardiovascular disease that causes important adverse circulatory events and is responsible of high mortality worldwide. Cortistatin is a neuropeptide produced by immune cells and expressed in the vascular system and atherosclerotic plaques that inhibits inflammation in different experimental models of autoimmune diseases, including atherosclerosis. Here, we investigated whether a deficiency in cortistatin predisposes to suffer exacerbated atherosclerosis by using established preclinical mouse models. We generated mice that lack of apolipoprotein E ($apoE^{-/-}$) and are totally ($CST^{-/-}$, KO) or partially ($CST^{+/-}$, HET) deficient in cortistatin. Mice were subjected to carotid partial ligation and fed a hyperlipidemic diet for four weeks to induce acute localized plaques, or alternatively were fed a normal or hypercholesterolemic diet for various weeks to induce chronic atherosclerotic plaques in aorta and aortic sinus. We observed that $apoE^{-/-}$CST-ko and $apoE^{-/-}$CST-het mice developed higher (number and size) atherosclerotic plaques in carotid artery, heart, aortic arch and aorta than $apoE^{-/-}$CST-wt mice fed a high-lipid diet. Interestingly, total or partial deficiency in cortistatin predisposed significantly to increased mortality during the progression of this disease.

Even with normal diet, apoE$^{-/-}$CST-ko and apoE$^{-/-}$CST-het mice showed early severe atherosclerotic plaques in heart and aortic arch. Lack of cortistatin did not change serum cholesterol, but increased the presence of lipid-loaded macrophages and of Th1 and Th17 cells in plaques and draining-lymph nodes and of inflammatory M1 macrophages in peritoneum. Our findings demonstrate the endogenous role of cortistatin in the regulation of and potential predisposition to inflammatory cardiovascular disorders.

P.C2.01.04

The effect of HLA on autoantibody isotypes & cytokine production in myasthenia gravis with autoantibodies to muscle specific tyrosine kinase (MuSK-MG)

M. CEBI[1], *H. DURMUS*[2], *S. YENTUR*[1], *V. YILMAZ*[1], *F. AYSAL*[3], *Y. PARMAN*[2], *P. OFLAZER*[2], *F. DEYMEER*[2], *G. SARUHAN-DIRESKENELI*[1];
[1]*ISTANBUL UNIVERSİTY, ISTANBUL MEDICINE FACULTY, DEPARTMENT OF PHYSIOLOGY, İSTANBUL, Turkey,* [2]*ISTANBUL UNIVERSİTY, ISTANBUL MEDICINE FACULTY, DEPARTMENT OF NEUROLOGY, İSTANBUL, Turkey,* [3]*Bakırköy Sadi Konuk State Hospital, İSTANBUL, Turkey.*

A small subset of myasthenia gravis (MG) develops with autoantibodies against muscle-specific kinase (MuSK). These anti-MuSK autoantibodies are predominantly of IgG4 isotype. MuSK-MG is strongly associated with *HLA-DQB1*05* with *HLA-DRB1*14* or *HLA-DRB1*16*. In this study, the effect of these HLA-associations on the anti-MuSK IgG autoantibody isotype and antibody-related cytokine production was investigated.
Among all patients with MG who were followed at the Neuromuscular Unit of the Department of Neurology of Istanbul Medical Faculty, 80 patients with anti-MuSK antibodies were selected. Disease-associated HLA types were detected in the collected DNA samples. Anti-MuSK-IgG1, -IgG2, -IgG3 and -IgG4 antibody titers and levels of IL-6, IL-17A and IL-10, measured in the sera by ELISA, compared between the groups with or without *HLA-DQB1*05, HLA-DRB1*14* or *HLA-DRB1*16* by non-parametric tests.
Anti-MuSK-IgG4 titers were significantly higher than -IgG1, -IgG2 and -IgG3 isotypes ($p< 0.0001$, $p< 0.0001$, $p< 0.0001$) in the whole group of patients. Anti-MuSK-IgG1 titers were also relatively higher than -IgG2 and -IgG3 titers ($p< 0.0001$, $p< 0.0001$). When the titers were compared between HLA subgroups, *DRB1*14 (+)* MuSK-MG patients had higher IgG4 and IgG1 titers than *DRB1*14 (-)* patients ($p= 0.017$, $p= 0.0002$). Higher IL-10 ($p= 0.048$) and lower IL-17A ($p= 0.011$) levels were measured in *DRB1*14* (+) patients compared to *DRB1*14 (-)* patients. No other differences were detected.
Higher titers of anti-MuSK-IgG4 and -IgG1 autoantibodies in *HLA-DRB1*14* (+) patients suggest the role of HLA in isotype switching. Differences of IL-17A and IL-10 in *HLA-DRB1*14* subgroups strengthen the role of *DRB1* in this autoimmune response.

P.C2.01.05

A diet high in fibre can moderate inflammation and kidney pathology in a model of systemic lupus erythematosus

T. A. Gottschalk*, E. Tsantikos, M. L. Hibbs;*
Monash University, Melbourne, Australia.

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease mediated by the deposition of immune complexes in tissues including the kidney, with the ensuing inflammatory cascade driving progressive tissue damage and dysfunction. Mice lacking Lyn tyrosine kinase (Lyn$^{-/-}$ mice) develop an autoimmune disease similar to SLE, driven by dysregulation of the immune system, immune complex deposition in tissue and systemic inflammation culminating in progressive glomerulonephritis. The gut microbiome has been shown to have an immunoregulatory effect on the development of autoimmune and inflammatory diseases, in large part due to the production of short chain fatty acids from the fermentation of dietary fibre. To determine whether dietary fibre could moderate systemic autoimmune and inflammatory pathology, Lyn$^{-/-}$ mice and control C57BL/6 mice were fed a high fibre diet (HFD) or a standard control diet from weaning until 42 weeks old. On the control diet, Lyn$^{-/-}$ mice developed lymphopenia, splenomegaly from enhanced splenic myelopoiesis, immune cell hyperactivation, and pathogenic IgG anti-dsDNA autoantibodies resulting in glomerulonephritis.
These hallmarks of inflammation and autoimmune disease were significantly moderated in HFD fed Lyn$^{-/-}$ mice, indicating that dietary intervention is effective at dampening chronic systemic inflammation and glomerular pathology. These findings highlights the contribution of diet and the gut microbiome in regulating systemic immune responses and controlling autoimmunity, inflammation, and preventing the progression of immunopathology and suggests that fibre supplementation may improve outcomes for those living with SLE or other chronic systemic inflammatory diseases. This work was funded by grants from the NHMRC of Australia and Monash CCS.

P.C2.01.06

CD11b regulates inflammation, autoimmunity and associated pathology in a model of systemic lupus erythematosus

T. A. Gottschalk*, E. Tsantikos, M. L. Hibbs;*
Monash University, Melbourne, Australia.

Systemic Lupus Erythematosus (SLE) is a complex, heterogeneous autoimmune disease characterized by circulating self-reactive antibodies that deposit in tissues including the kidneys, alongside a chronic inflammatory response that leads to progressive tissue damage and impaired function. Genome-wide association studies have identified a number of receptors and signal transduction molecules specific for the immune system that predispose to the development of SLE. A loss-of-function single nucleotide polymorphism in the *Itgam* gene encoding leukocyte integrin CD11b (rs1143679) has been identified which associates with an increased incidence of SLE, implicating CD11b as a protective factor against disease development. To understand the role that CD11b plays in controlling autoimmune disease, we crossed CD11b deficient mice (CD11b$^{-/-}$) with Lyn deficient (Lyn$^{-/-}$) mice, a well-studied, robust model of human SLE. Double knockout Lyn$^{-/-}$CD11b$^{-/-}$ mice were analysed over time for development of autoimmune disease and inflammation. While aged mice lacking CD11b alone did not develop autoimmune disease, deficiency of CD11b on the Lyn-deficient autoimmune-prone background exacerbated disease, driving splenomegaly and lymphadenopathy, extramedullary haematopoiesis, autoantibody production and glomerulonephritis, which heavily impacted survival. These findings confirm that CD11b is an autoimmune susceptibility gene that when mutated can exacerbate the severity of disease on a susceptible genetic background. This work highlights an important role for CD11b in regulating and controlling the progression of inflammation and autoimmune disease. This work was funded by grants from the NHMRC of Australia and Monash CCS.

P.C2.01.07

Integration of genome-wide DNA methylation and transcription uncovered aberrant methylation-regulated genes and pathways in the peripheral blood mononuclear cells of systemic sclerosis

Q. Li[1], *H. Zhu*[2], *C. Zhu*[1], *W. Mi*[1], *H. Luo*[2], *X. Zuo*[2];
[1]*University of Texas Southwestern Medical Center, Dallas, United States,* [2]*Xiangya Hospital, Central South University, Changsha, China.*

Objectives: The aim is to delineate the interaction network between gene transcription and DNA methylation in PBMC of systemic sclerosis (SSc) patients and to identify methylation-regulated genes involved in the pathogenesis of SSc. Methods: Genome-wide mRNA transcription and global DNA methylation analysis were performed on PBMC from 18 SSc patients and 19 matched normal controls (NC) using Illumina BeadChips. Differentially expressed genes (DEGs) and differentially methylated positions (DMPs) were integratively analyzed to identify methylation-regulated genes and associated molecular pathways. Results: Transcriptome analysis distinguished 453 DEGs (269 up- and 184 down-regulated) in SSc from NC. Global DNA methylation analysis identified 925 DMPs located on 618 genes. Integration of DEGs and DMPs revealed 20 potential methylation-regulated DEGs (MeDEGs), including 12 up-regulated genes (ELANE, CTSG, LTBR, C3AR1, CSTA, SPI1, ODF3B, SAMD4A, PLAUR, NFE2, ZYX and CTSZ) and eight down-regulated genes (RUNX3, PRF1, PRKCH, PAG1, RASSF5, FYN, CXCR6 and F2R), are predominantly involved in the migration, proliferation, activation and inflammation of immune cells. Support vector machines analysis identified six out of the 20 MeDEGs, including F2R, CXCR6, FYN, LTBR, CTSG and ELANE, which clearly distinguished SSc from NC with 100% accuracy. The expression for four MeDEGs, F2R, FYN, PAG1 and PRKCH, significantly decreased in SSc with interstitial lung disease (ILD) compared with NC or SSc without ILD. Conclusion: The identified MeDEGs may represent novel candidate factors in the etiology and pathology of SSc and may lead to the abnormal activation of immune regulatory pathways and potentially contribute to the pathogenesis of SSc.

P.C2.01.08

C1q restrains autoimmunity and viral infection by regulating CD8$^+$T-cell metabolism

G. Ling[1], *G. Crawford*[1], *N. Buang*[1], *I. Bartok*[1], *K. Tian*[1], *N. Thielens*[2], *I. Bally*[3], *J. Harker*[1], *P. Ashton-Rickardt*[1], *S. Rutschmann*[1], *J. Strid*[1], *M. Botto*[1];
[1]*Imperial College London, London, United Kingdom,* [2]*University Grenoble Alpes, Grenoble, France,* [3]*University Grenoble Aples, Grenoble, France.*

Deficiency of complement C1q, the first component of the classical pathway, is strongly associated with the development of systemic lupus erythematosus (SLE). Explaining this strong association in terms of abnormalities in the classical complement pathway alone remains problematic because C3 deficiency does not predispose to SLE. Here we demonstrate, using a chronic graft-versus-host-disease (cGvHD) lupus model, that C1q, but not C3, restrains the immune response to self-antigens by controlling effector CD8$^+$ T cells. In the absence of C1q, the turnover of the memory precursor effector CD8$^+$T cells was accelerated because the mitochondrial spare respiratory capacity, which provides bioenergetic advantage for survival, was reduced. This C1q-mediated metabolic effect favoured the expansion of the short-lived effector CD8$^+$T cells which are the main source of granzyme B that can generate unique autoantigen fragments. Furthermore, depletion of CD8$^+$T cells limited the propagation of the autoimmune response in cGvH-induced *C1qa*$^{-/-}$mice, indicating the direct contribution of these cells to the disease progression. Consistent with the findings in the SLE-cGvHD model, C1q deficiency also triggers an exuberant effector CD8$^+$ T-cell response to chronic lymphocytic choriomeningitis virus infection leading to lethal immunopathology. Our results demonstrate that C1q, independently of complement activation, operates as a key determinant in the development of a balanced effector CD8$^+$ T response.These data establish a link between C1q and CD8$^+$T-cell metabolism and may explain how C1q protects against lupus, with implications for the role of viral infections in the perpetuation of autoimmunity.

P.C2.01.09

Exploiting annexin-mediated immunosuppression to induce antigen-specific tolerance

***C. S. Link**, F. Bujupi, H. Weyd, P. H. Krammer;*
German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany.

Apoptotic cells mediate immunosuppression of dendritic cells (DC) and inhibit immune responses. Thereby, apoptotic cells facilitate the induction of peripheral tolerance and the prevention of autoimmune diseases. We investigate the influence of apoptotic cells on DC and identified the cell surface exposure of the evolutionary conserved annexin core domain (Anx) as a specific signal, which binds to specific receptors on DC and antagonises Toll-like receptor signalling. To further test this tolerogenic capacity of Anx for potential downregulation of antigen-specific immune responses, we generated beads harbouring Anx as well as the model antigen ovalbumin (OVA). Treating BMDC with either Anx-OVA beads (OAB) or with OVA-beads (OB) as a control and subsequent co-culture with OVA-specific $CD4^+$ T cells, we could show diminished T cell activity in the presence of Anx. Furthermore, Anx leads to reduction of cytokine secretion (IL-2, IFN-γ) and of proliferation that suggests induction of T cell anergy by the OAB treatment. These results indicate that coupling of Anx and a defined antigen or allergen in a therapeutic bead preparation might be used as a new approach to downregulate pathologic immune responses in the context of autoimmunity and allergy.

P.C2.01.10

Selective inhibition of gelatinases in CD4+ T-cells reduces clinical severity in a murine model of multiple sclerosis

***L. Onwuha-Ekpete**, D. Tokmina-Roszyk, G. B. Fields;*
Florida Atlantic University, Jupiter, United States.

Introduction: MMP-2 and MMP-9 are the gelatinase members of the matrix metalloproteinase (MMP) family of proteolytic enzymes that mediate the degradation of extracellular matrix components. MMPs are essential for normal physiological processes, but their dysregulation is associated with various pathologies including multiple sclerosis (MS). Experimental autoimmune encephalomyelitis (EAE) is a well-established murine model of MS. In EAE, CD4+ T-cells activated in the periphery penetrate the blood-brain barrier (BBB), recruit immune cells, initiate destruction of the myelin sheath, and cause axonal loss. The gelatinases are required for these various processes. Recent studies have implicated the gelatinases in normal T-cell activation; however, the mechanism of action is not known. Materials and Methods: For *in vitro* assays, CD4+ T cells were activated with CD3/CD28 mAb beads after prior treatment with a gelatinase inhibitor. For *in vivo* assays, EAE mice were treated daily from Day 7 with vehicle or inhibitor and disease progression monitored. Results: Upon activation, CD4+ T-cells treated with inhibitor demonstrated a reduced ability to enter cell cycle, proliferate, and produce cytokines. In addition, RNA-seq analysis highlighted key proteins associated with the T-cell receptor signaling pathway that are impacted. Furthermore, treatment of EAE mice with an MMP-2/MMP-9 inhibito resulted in reduced clinical severity. Conclusion: In our studies, we demonstrated that the gelatinases are important for homeostatic maintenance as well as a robust antigenic stimulation. These results emphasize the importance of gelatinases as therapeutic targets in CD4+ T-cell mediated autoimmune diseases.

P.C2.01.11

Hyperglycemia-dependent NF-kappaB O-GlcNAcylation acts as a molecular switch regulating T cell and T reg cell function in autoimmunity

***P. Ramakrishnan**, T. de Jesus, L. Liu;*
Case Western Reserve University, Cleveland, United States.

Type 1 diabetes is an autoimmune disease associated with hyperglycemia. Adverse pathological effects of hyperglycemia include posttranslational modification of proteins by the sugar N-acetyl glucosamine (GlcNAc) in a process called O-GlcNAcylation. We found that hyperglycemia induces O-GlcNAcylation of NF-kappaB protein c-Rel, which is a critical regulator of T cell function and T regulatory cell development. O-GlcNAcylation of c-Rel at serine 350 activates c-Rel-dependent transcription of proautoimmune cytokines and inhibits the expression of T regulatory cell specific transcription factor FOXP3. Thus, c-Rel O-GlcNAcylation may serve as a key regulatory switch with dual, but reciprocal, roles in positively regulating autoimmune T cell and negatively regulating immunosuppressive T regulatory cell function. The net result of these two opposing effects may exacerbate autoimmunity in type 1 diabetes. This study reveals c-Rel O-GlcNAcylation as a disease-dependent novel molecular mechanism regulating autoimmunity and a potential therapeutic target to control autoimmunity in type 1 diabetes.

P.C2.01.12

High-throughput screening for Lck-coreceptor coupling inhibitor

***K. Ruppova**, V. Horkova, O. Stepanek;*
Institute of Molecular Genetics of the ASCR, Prague, Czech Republic.

TCR-mediated activation of T lymphocytes is the key event for the initiation of adaptive immune response that is directed against pathogens but also against self-antigens in the case of autoimmune diseases. The initiation of TCR signaling is promoted by coreceptors CD4 and CD8 that mediate the interaction of TCR signaling complex with Lck kinase which triggers downstream signaling. We developed a functional model to study Lck-coreceptor coupling. Our data show that Lck-coreceptor coupling is especially important for T cell activation by low affinity antigens and that T cell activation increases with increased Lck-coreceptor coupling. Moreover, it was shown that, in contrast to foreign antigens, self-antigens are recognized with low affinity by TCR of autoreactive T cells that escaped negative selection in thymus. Thus, we assume that inhibiting of Lck-coreceptor interaction should prevent activation of autoreactive T cells that are the main cause of autoimmune diseases while leaving the protective immunity against foreign antigens unaffected.
The association of Lck and coreceptor is mediated by a unique protein-protein interaction where two cysteines from each partner are coordinated by a zinc cation. Thus, we will perform a high-throughput screening to find an inhibitor of this interaction using peptides derived from Lck and CD4 or CD8. Candidate molecules will be tested to inhibit the interaction in living cells using FRET technique and later to inhibit TCR signaling using luciferase reporter system. Selected molecule(s) will be finally tested to inhibit autoimmunity in mouse models.

P.C2.01.13

Thymol blunts experimental autoimmune orchitis-induced reproductive failure in BALB/c mice

*E. Yarahmadi, **A. Shalizar-Jalali**, G. Najafi, M. Abtahi-Foroushani;*
Faculty of Veterinary Medicine, Urmia University, Urmia, Iran, Islamic Republic of.

Introduction: Autoimmune orchitis as an autoimmune aggression to testis can result in male subfertility and/or infertility. This study was aimed to scrutinize the possible repro-protective activity of thymol (TML) against experimental autoimmune orchitis (EAO)-induced alterations in testicular histoarchitecture and epididymal sperms characteristics and *in vitro* fertilizing potential in mice.
Materials and Methods: In this experimental study, 36 adult male BALB/c mice were randomly categorized to six equal groups including untreated, TML (100 $mgkg^{-1}$BW/day; orally for 5 weeks), antigen (100 µl; subcutaneously) and *Bordetella pertussis* (10^9 bacteria at the day of antigen injection and 48 hours later; intraperitoneally) control groups, EAO and EAO + TML. The EAO was induced through testicular homogenate plus complete Freund's adjuvant plus *B. pertussis* injection. All animals were euthanized after 5 weeks and testicular histoarchitecture as well as epididymal sperms characteristics and *in vitro* fertilizing ability were analyzed.
Results: The EAO caused significant reductions in the seminiferous tubules diameter, germinal epithelium height, quality and maturation along with severe testicular morphological alterations including spermatogenic cells maturation arrest, seminiferous tubules depletion and also multinuclear giant cells formation compared to control, TML, antigen and *B. pertussis* groups. Moreover, EAO resulted in epididymal sperms quantity, quality and fertilizing potential reduction as well as embryo development impairment. While, TML co-administration led to remarkable amelioration of EAO-induced reproductive disorders.
Conclusions: These findings revealed that TML has repro-protective activities against EAO-evoked disorders in male mice reproductive system.
This study was funded by Urmia University, Urmia, Iran.

P.C2.01.14

Paquinimod prevents development of diabetes in the non-obese diabetic (NOD) mouse

***S. Tahvili**[1], M. Törngren[2], D. Holmberg[1], T. Leanderson[1], F. Ivars[1];*
[1]Lund university, Lund, Sweden, [2]Active Biotech AB, Lund, Sweden.

Introduction: Quinoline-3-carboxamides (Q compounds) are immunomodulatory compounds that have shown efficacy both in autoimmune disease and cancer. We have in here investigated the impact of one such compound, paquinimod, on the development of diabetes in the NOD mouse model for type I diabetes (T1D).Methods and Results: In cohorts of NOD mice treated with paquinimod between weeks 10 to 20 of age and followed up until 40 weeks of age, we observed dose-dependent reduction in incidence of disease as well as delayed onset of disease. Further, in contrast to untreated controls, the majority of NOD mice treated from 15 weeks of age did not develop diabetes at 30 weeks of age. Importantly, these mice displayed significantly less insulitis, which correlated with selectively reduced number of splenic macrophages and splenic $Ly6C^{hi}$ inflammatory monocytes at end point as compared to untreated controls. Conclusion: Collectively, these results demonstrate that paquinimod treatment can significantly inhibit progression of insulitis to T1D in the NOD mouse. We propose that the effect of paquinimod on disease progression may be related to the reduced number of these myeloid cell populations. Our finding also indicates that this compound could be a candidate for clinical development towards diabetes therapy in humans.

P.C2.01.15

Astrocytic ShcC/Rai supports the function of autoreactive T cells during EAE by modulating adenosine-dependent CTLA4 expression through the inhibition of CD39 and CD73 activity

C. Ulivieri[1], *D. De Tommaso*[1], *F. Finetti*[1], *B. Ortensi*[2], *G. Pelicci*[3], *M. D'Elios*[4], *C. Ballerini*[4], *C. T. Baldari*[1];
[1]*University of Siena, Siena, Italy,* [2]*European Institute of Oncology, Milano, Italy,* [3]*University "Amedeo Avogadro" Novara, Novara, Italy,* [4]*University of Florence, Florence, Italy.*

Autoreactive T cell recruitment and activation in the central nervous system (CNS) are two recognized pathogenic processes in Multiple Sclerosis (MS). Recent data indicate, however, that the interplay between cells resident in the CNS, including astrocytes, and infiltrated T cells is instrumental for disease onset and progression. In this context, while the impact of astrocytes on the T cell dependent autoimmune response has been partially addressed, how autoreactive T cells modulate astrocytes during experimental autoimmune encephalomyelitis (EAE) has not been explored. We have recently demonstrated that ShcC/Rai is as a novel astrocytic adaptor whose loss in mice accounts for a milder EAE notwithstanding a higher frequency of CNS infiltrated autoreactive T cells. Here we have explored and characterized the molecular mechanism that underlies the reciprocal modulation of astrocytes and autoreactive T cells, focusing on the role of ShcC/Rai. We found that astrocytes respond to autoreactive T cellsinjury by upregulating the ectonucleotidases CD39 and CD73 through both contact-dependent and -independent mechanisms. We also demonstrate that Rai dampens the enzymatic activity of CD39 and CD73 in astrocytes, thereby preventing the degradation of pro-inflammatory extracellular ATP to its immunosuppressive metabolite adenosine and hence supporting the pathogenic potential of autoreactive T cells. Accordingly, we found that the microenvironment shaped by Rai deficient astrocytes inhibited T cell proliferation and TCR signaling more efficiently compared with control astrocytes by promoting adenosine-dependent CTLA4 upregulation in recently activated T cells. Collectively, we have identified a new mechanism by which astrocytes sustain the pathogenic potential of autoreactive T cell.

P.C2.01.16

New insights into Cyclin D3 signaling involved in beta cell wellness and survival

C. Vived*, C. Santos-Rosendo, M. de la Torre, L. Egia-Mendikute, M. Corral-Pujol, E. Rosell-Mases, J. Verdaguer, C. Mora;*
University of Lleida/IRB Lleida, Lleida, Spain.

Introduction: Autoimmune diabetes is caused by the destruction of insulin producing pancreatic beta cells. Cyclin D3 is involved in CDK-dependent cell cycle progression. Nevertheless, our group has reported that cyclin D3, which is downregulated in beta cells upon inflammation, is essential for protecting beta cells in front of the inflammation-induced apoptosis and for maintaining proper function of beta cells, both in a cell-cycle independent fashion. Materials and Methods: We have identified by the yeast two-hybrid technology (Y2H) a number of molecules other than the CDKs that physically interact with cyclin D3 to unveil potential signaling pathways responsible for the protective role of cyclin D3 in front of the autoimmune insult. Results: We have observed that cyclin D3 interacts with proteins involved in diverse physiological processes. We are assessing the physical interaction between cyclin D3 and the different candidates in eukaryotic cell models by different experimental approaches. Conclusion: We focus on the molecules obtained from Y2H that are not involved in the cell cycle, in order to dissect metabolism and viability and cell cycle. In this way, we can analyse this unknown role of cyclin D3 in the viability and fitness of beta-pancreatic cells and it helps the translation into future therapeutic targets for T1D.

P.C2.01.17

Trained autoimmunity as a driver in the pathogenesis of Systemic Lupus Erythematosus

C. Yanginlar*, N. Rother, J. van der Vlag;*
RIMLS, Radboudumc, Nijmegen, Netherlands.

Introduction: Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by autoantibodies against chromatin. Elevated levels of circulatory chromatin are detected in SLE patients, which may be a result of aberrancies in apoptosis or neutrophil extracellular trap (NET) formation or insufficient clearance of apoptotic material or NETs. Recently, we showed that SLE-derived PBMCs appeared more sensitive to apoptotic microparticles (MPs) than those from controls, for which there is no clear explanation yet. Recently the concept of trained immunity was described, meaning that innate immune cells can develop a memory after first exposure to disease/pathogen associated molecular patterns (DAMPs/PAMPs) which results in a stronger response after subsequent exposures. We hypothesized that sources of nuclear antigens in SLE, including MPs and NETs, can train PBMCs, thereby inducing trained autoimmunity.
Methods: PBMCs from healthy volunteers were trained 24 hours with different stimuli (untrained, heat killed *C. albicans*, MPs, NETs). After 6 days of resting, cells were stimulated with TLR2 (Pam3CSK4) or TLR4 (LPS:B5) antagonists for 24 hours and IL-6 and TNF-α levels were measured.
Results: NETs induced PBMC training dose dependently, which was based on secretion of higher levels of IL-6 and TNF-α in response to LPS:B5 or Pam3CSK4 after the resting period. Training by NETs was comparable to that induced by *C. albicans*. MPs, on the other hand, induced training at a lesser extent.
Conclusions: Innate immune cells can be trained by MPs and NETs, which may play an important role in the pathogenesis of SLE and lupus nephritis.
CY: Radboudumc PhD-Fellowship

P.C2.01.18

Dysregulated T cell activity in systemic lupus erythematosus

H. Zhou, T. Wu, J. LI, B. LI, ***F. Yu****;*
Affiliated Hospital of Guizhou Medical University, Guiyang, China.

Background: Accumulating evidence indicates a critical role for T lymphocytes and relavant cytokines in the pathogenesis of Systemic lupus erythemotosus (SLE). However, the specific contribution of T lymphocytes together with the related circulating cytokines in disease pathogenesis and organ involvement is still not clear. Methods: Blood samples were collected from 49 SLE patients and 22 healthy controls (HC). Expressions of HLA-DR and co-stimulatory molecules on T cells were evaluated by flow cytometry. Concentrations of serum C-reactive protein, erythrocyte sedimentation rate, anti-double-stranded DNA (anti-dsDNA) antibody, total IgG, complement 3, complement 4 were measured. Serum cytokines and chemokines were measured by a cytometric beads array assay. Results: Elevated frequencies of HLA-DR$^+$T cells and ICOS$^+$T cells were observed in SLE patients with positive anti-dsDNA antibodies compared with those in HC. The expression of HLA-DR$^+$T cells was positively correlated with SLEDAI. Furthermore, levels of serum IL-6, MCP-1, TNFRI, IL-10 and IL-12 were detected higher in SLE patients compared with HC. In addition, patients with hematologic manifestations displayed elevated frenquencies of HLA-DR$^+$-, ICOS$^+$- but lower TIGIT$^+$CD4$^+$-T cells. Patients with renal manifestations displayed decreased levels of serum CCL20 and MCP-1 but an increased frenquency of HLA-DR$^+$T cells. Conclusion: SLE subjects exhibited dysregulated T cell activity and the cytokine expression profile. Furthermore, we developed a chemokine and cytokine profiling strategy to predict the activity of SLE, which has clinical implication for monitoring the flares and remission during the course of SLE in order to improve clinical outcomes.

P.C2.01.19

A novel animal model for systemic sclerosis induced by immunization ofangiotensin II receptor 1

X. Yue[1], *F. Petersen*[1], *X. Wang*[1], *J. Yin*[1], *H. Heidecke*[2], *G. Wallukat*[3], *S. Ingolf*[3], *A. Philippe*[4], *D. Dragun*[4], *G. Riemekasten*[5], *X. Yu*[1];
[1]*Research center borstel, Borstel, Germany,* [2]*CellTrend GmbH, Luckenwalde, Germany,* [3]*Berlin Cures GmbH, Berlin, Germany,* [4]*Department of Nephrology and Critical Care Medicine, Berlin, Germany,* [5]*University Hospital Lübeck, Lübeck, Germany.*

Background and Objectives: Systemic sclerosis (SSc) is a complex connective tissue disease which is characterized by autoimmunity, vasculopathy and fibrosis. Our recent study showed that the progression of SSc was strongly associated with the autoantibodies against angiotensin II receptor I (AT1R), suggesting a role of autoimmunity to AT1R in the pathogenesis of the disease. In this study, we aimed to investigate the role of AT1R in the pathogenesis of SSc in mice. **Methods:** C57BL/6J mice were immunized with membrane extract (ME) of CHO cell overexpressing human AT1R or with ME of CHO cells as control. Serum, lung and skin samples were collected and assessed 63 days after immunization for autoantibody production, inflammation and fibrosis, which are hallmarks for SSc. **Result:** Immunization with hAT1R induced the production of autoantibodies against the receptor in mice, and autoantibody deposition was found in the lung.
Histologically, mice immunized with hAT1R showed a SSc-like disease, including perivascular infiltrates and fibrosis in the skin as well as pulmonary inflammation. The inflammation in the skin and the lung were characterized by infiltration of T- and B-cells. **Conclusion:** This study demonstrates that immunization with hAT1R can induce a SSc-like disease, thus showing a pathogenic role of autoimmunity to AT1R and providng a novel mosue model for the diseases. Furthermore, this study also introduces a new immunization strategy to generate functional autoantibodies against receptors on the cell membrane.

P.C2.01.20

INS1007, a reversible dipeptidyl peptidase 1 inhibitor, reduces human neutrophil membrane-bound proteinase 3 expression and neutrophil serine protease activities

***J. Zhang**, J. Basso, D. LaSala, W. R. Perkins, K. DiPetrillo;*
Insmed Incorporated, Bridgewater, NJ, United States.

Introduction: Granulomatosis with polyangiitis (GPA) is a form of vasculitis characterized by necrotizing granulomatous inflammation. In most patients, this autoimmune disease is associated with anti-neutrophil cytoplasmic antibodies binding to membrane-bound proteinase 3 (mPR3) on neutrophils and stimulating degranulation and release of neutrophil serine proteases (NSPs) that damage tissues. Dipeptidyl peptidase 1 (DPP1) is a key enzyme that converts pro-PR3 to an active form. We hypothesized that inhibiting DPP1 activity with INS1007 could decrease mPR3 expression and NSP activities in neutrophils.
Methods: Human stem cells from either cord blood (CB) or bone marrow (BM) were differentiated into neutrophils *in vitro* in the presence of increasing INS1007 concentrations. mPR3 expression was assessed qualitatively by fluorescence microscopy and quantitatively by flow cytometry. Activities of NSPs, including neutrophil elastase (NE) and PR3, were measured in enzymatic assays using exogenous peptide substrates.
Results: Compared to untreated differentiated neutrophils, INS1007 concentration-dependently (0.000153 -10 μM) reduced both mPR3 levels and the percentage of cells expressing detectable mPR3. Additionally, INS1007 concentration-dependently decreased both PR3 (CB EC_{50} = 0.70 μM, BM EC_{50} = 0.28 μM) and NE (Both EC_{50} = 0.36 μM) activities in differentiated neutrophils, with greater than 90% reductions of enzyme activities at 10 μM.
Conclusions: INS1007 effectively lowered mPR3 expression and reduced NSP activities in *in-vitro*-differentiated neutrophils. Considering the central role of mPR3 as the autoantigen in GPA and the tissue damage and inflammation resulting from NSP activities, the multiple mechanisms by which INS1007 affects neutrophil function make it a potential therapy for treating GPA patients.

P.C2.02 Immune signaling and therapy in autoimmunity - Part 2

P.C2.02.01

Systemic immunophenotyping and cytokine profiling reveals inflammatory signature of alopecia areata

***K. Bain**[1], E. McDonald[1], I. McInnes[1], S. Holmes[2], A. Astrand[3], S. W. Milling[1];*
[1]Intistute of Infection, Immunity and Inflammation, Glasgow, United Kingdom, [2]Queen Elizabeth University Hospital, Glasgow, United Kingdom, [3]AstraZeneca, Gothenburg, Sweden.

Alopecia Areata (AA) is a T cell mediated autoimmune disease causing hair loss. AA can have profound psychological effects on affected individuals. Therapeutic options are limited, and a positive response is often followed by relapse upon treatment cessation. JAK inhibitors offer promise, but may cause significant immunosuppression. Human and murine studies have implicated $CD8^+NKG2D^+$ T cells in hair follicle damage, but little is known about the specific pathways and mediators involved in promoting and sustaining this response. Peripheral blood was obtained from consented volunteers at our dedicated research clinic. Peripheral blood mononuclear cells (PBMCs) were analysed using 11-parameter flow cytometry to identify $CD4^+$ T cell, $CD8^+$ T cell, dendritic cell (DC), natural killer (NK) cell and B cell populations. Multiplex analysis was performed to determine the plasma concentrations of inflammatory cytokines. Flow cytometric immunophenotyping revealed a significant increase in the number of circulating $CCR6^+$ $CD4^+$ T cells. We also observed altered frequencies of $CD4^+CXCR3^+$ T cells expressing the skin homing marker cutaneous lymphocyte antigen (CLA). Importantly, these changes are enhanced in participants with more extensive hair loss (alopecia totalis and alopecia universalis) evaluated by SALT score. Multiplex cytokine profiling revealed a strong inflammatory signature, characterised by a significant increase in the levels of circulating IL-17A, IL-33, IL-25, TNFalpha and IL-6. We have generated the first comprehensive immunophenotype in AA, and have not only discovered changes in circulating $CD4^+$ T cell populations, but have also revealed a strong systemic inflammatory/Th17 signature in these individuals.

P.C2.02.02

Functional elimination of autoreactive T and B cells by anti-annexin A1 antibody therapy in MRL/lpr murine model of systemic lupus erythematosus

***S. Bradyanova**[1], N. Mihaylova[1], P. Chipinski[1], S. Chausheva[1], Y. Manasiev[2], M. Herbáth[3], D. Kyurkchiev[4], J. Prechl[3], A. Tchorbanov[1,5];*
[1]Laboratory of Experimental Immunology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria, [2]Department of General Microbiology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria, [3]MTA-ELTE Immunology Research Group, Hungarian Academy of Sciences, Budapest, Hungary, [4]Laboratory of Clinical Immunology, University Hospital 'Sv.I.Rilski', Medical University Sofia, Sofia, Bulgaria, [5]National Institute of Immunology, Sofia, Bulgaria.

Introduction: Systemic lupus erythematosus is an autoimmune syndrome characterized by the development of autoantibodies to a wide range of antigens and multiple organ involvement. Together with B cells, respective self-reactive T cells have an important contribution in disease progression as being responsible for inflammatory cytokines secretion, B cell activation, and promoting amplification of the autoimmune and inflammatory response. Annexin A1 is expressed by many cell types and binds to phospholipids in a Ca^2+ dependent manner. Abnormal expression of annexin A1 was found on activated B and T cells in both murine and human autoimmunity suggesting its potential role as a therapeutic target. Materials and Methods: Groups of lupus-prone MRL/lpr mice were treated with an anti-annexin A1 monoclonal antibody and the disease activity and survival of the animals were monitored. ELISA and ELISpot assays, RT-PCR, cell proliferation assay, flow cytometry, histological and immunofluorescence kidney analyses were used to determine the levels of cytokines, anti-dsDNA antibodies and kidney injuries. Results: Administration of anti-annexin A1 monoclonal antibody resulted in suppression of IgG anti-dsDNA antibody production and of proteinuria, modulation of cytokine production, improved kidney histology, decreased disease activity and prolonged survival compared to the control group. Conclusions: The anti-ANX A1 antibody therapy described here obviously targets over-activated autoreactive cells and has a beneficial effect on earlier stage of lupus development. The administration of ANX A1 antibody strongly suppresses the ongoing autoimmune disease in lupus-prone MRL/lpr mice and by using such a therapy it is possible to down-regulate the activity of lupus-associated lymphocytes.

P.C2.02.03

Transcriptomic analysis of CD4+ and CD8+ T cells from lupus nephritis patients clustered them into type I IFN-high and IFN-low expressing patients irrespective of their disease activity

***N. B. Buang**, G. Ling, L. Stephens, F. Doyle, M. Pickering, M. Botto;*
Imperial College London, London, United Kingdom.

Systemic Lupus Erythematosus (SLE) is a relapsing-remitting autoimmune disease and we lack biological parameters with which to monitor and predict disease flares. Recent studies have postulated that exhaustion signatures from CD8+ T cells can be used as a biomarker to predict long-term prognosis in SLE. To investigate if the T cell transcriptomic signatures can be utilised to define disease activity, mRNA from CD4+ and CD8+ T cells from active (SLEDAI > 4, n=12) and inactive (SLEDAI < 4, n=16) Lupus Nephritis (LN) patients was sequenced and correlated with 84 clinical criteria. Principle Component Analysis shows overlapping global gene expression between active and inactive LN patients. Unsupervised hierarchical clustering of all differentially expressed genes between LN patients and healthy controls grouped patients into two groups: individuals expressing high type I Interferon (IFN) (active LN n=8, inactive LN n=6) and those with low IFN signatures (active LN n=4, inactive LN n=10). No difference in SLEDAI, BILAG, and anti-dsDNA levels could be observed between the 2 IFN groups. Gene Set Variation Analysis identified larger gene sets (200) to correlate with disease activity in CD8+ T cell gene signatures compared to CD4+ T cells (59), indicating CD8+ T cell signatures may be more informative to predict disease activity than CD4+ T cells. These signatures are involved in cell cycle, peroximl lipid metabolism, mitochondrial and proteasome pathways. Furthermore, there was no correlation between disease activity and the degree of the IFN signatures indicating that IFN may not play a key role in driving LN flares.

P.C2.02.05

Treg-of-B cells attenuated the progression of primary biliary cholangitis via modulating the activation of antigen-presenting cells

***Y. Chen**, B. Chiang;*
Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan.

Introduction. Primary biliary cholangitis (PBC), a liver-specific autoimmune diseases, is characterized by the presence of antimitochondrial antibody (AMA) and chronic progressive destruction of intrahepatic bile ducts with infiltrating mononuclear cells. Regulatory T cells (Tregs) attenuated the maturation of antigen-presenting cells (APCs) which play an important role to initiate immune responses. ducts with infiltrating mononuclear cells in the portal tract. chronic progressive destruction of small intrahepatic bile ducts with infiltrating mononuclear cells in the portal tract. chronic progressive destruction of small intrahepatic bile ducts with infiltrating mononuclear cells in the portal tract. Materials and Methods. In our study, splenic B cells without additional cytokines or chemicals induced the generation of Treg-like cells which referred to as Treg-of-B cells for the treatment of PBC. Results. Treg-of-B cells suppressed the proliferation of T cells, showed high expressions of LAG3 and CTLA-4, without expressing Foxp3. After incubation with Treg-of-B cells for 24 hr, LPS treated dentritic cells reduced productions of IL-6 and TNF-α and expressions of costimulatory molecules (CD80 and CD86). The inhibitory effect was dependent on cell-cell contact manner via CTLA-4 pathway. In mouse model of PBC, Treg-of-B cells were intravenously injected during the progression of disease. The level of AMAs, the cell infiltrations of APCs and lymphocytes, and the degree of fibrosis were significantly reduced in Treg-of-B cells treated group. Conclusion. Our results demonstrated that Treg-of-B cells can alleviate the inflammatory processes of PBC. It is suggested that Treg-of-B cells might be a potential therapeutic approach for autoimmune disease.

P.C2.02.06

The HLA class II risk genes associated to celiac disease are preferentially expressed compared to non-associated HLA genes either in *cis* and in *trans* configuration: implication for the pathogenic anti-gluten T-cell response

*C. Gianfrani[1], L. Pisapia[2], S. Picascia[2], F. Farina[2], R. Spettrini[2], **G. Del Pozzo**[2];*
[1]Instiitute of Protein Biochemistry-CNR, Naples, Italy, [2]Instiitute of Genetics and Biophysics-CNR, Naples, Italy.

Introduction We have recently studied the expression of DQA1*05 and DQB1*02 alleles, encoding the DQ2.5 molecule presenting the gluten peptides. We discovered that specialized Antigen Presenting Cells (APC) either DR3-DR3 homozygous or DR1-DR3 heterozygous show similar level of DQ2.5 transcripts and surface molecules. As a consequence, DQ2.5 APC induced similar activation level of gluten-specific T lymphocytes (DOI: 10.1016/j.jaut.2017.12.0). **Methods** We measured the quantity of DQA1* and DQB1* mRNAs by RT-qPCR, the DQα1*05 and DQβ1*02 surface proteins in APC by flow cytometry and the magnitude of gluten-specific $CD4^+$ T cell activation by INFγ ELISA. **Results** We found that DQA1*05 and DQB1*02 risk alleles were more expressed than non CD-predisposing alleles also in B cells with DQ2.5 genes in *trans* (DR5/DR7) configuration. The differential expression between CD-associated and non-associated alleles was significant also in the APC from non-affected subjects, although in these latters the increments was much lesser than in CD patients. The quantification of DQα1*05 and DQβ1*02 proteins by specific monoclonal antibodies confirmed these findings. Furthermore, we assessed that B cells carrying the DQ2.5 genes in *trans* and in *cis* configurations induce a comparable activation of specific $CD4^+$ T cell response to gluten epitopes. **Conclusions** Our findings indicated that the high level of HLA class II risk genes expression, independently by the gene dosage, strongly influence immune response against gluten in the intestinal mucosa of subjects with CD. Our results have a general relevance as the high expression of predisposing HLA alleles may favour the establishment of autoimmunity.

P.C2.02.07

Clinical manifestations and anti-TNF alpha treatment of juvenile Behçet's disease in Taiwan: A retrospective study.

***Y. Hu**, Y. Lin, Y. Yang, B. Chiang;*
Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan.

Background. Behçet's disease (BD) is a rare vasculitic disorder affecting all sizes of vessels. Patients with diagnosed age younger than 16 years old is defined as juvenile BD, which only accounts for 4 to 25 percent among all BD patients. This study aimed to evaluate the incidence change, clinical manifestation and treatment including anti-TNF alpha agents of patients with juvenile BD patients in Taiwan.
Methods. We retrospectively reviewed the patients younger than 16 years old with diagnosis of Behçet's disease in National Taiwan University Children's Hospital between 2008 to 2017.
Results. Total 66 patients were included in the study. The mean age at onset was 10.4 4.2 years. The most common clinical presentation was recurrent oral aphtosis (98.5%), which was also the most common initial symptoms. The most frequently used treatments were colchicine (57.6%) and systemic steroid (66.7%). Among 66 patients, 6 of them received anti-TNF alpha treatments due to refractory or severe BD. The BD patients with anti-TNF alpha agents have younger age at disease onset (7.0 v.s. 11 years, p=0.027) and diagnosis, (7.5 v.s. 13 years, p=0.012). All of the 6 patients were free of steroid use at the 1st year after treatment. Anti-TNF alpha therapy was well tolerated in all cases.
Conclusions. Juvenile BD was a rare disease but the incidence seemed increased recently. Patients with younger age, presenting gastrointestinal symptoms and arthritis might tend to have more severe disease. Anti-TNF alpha therapy might be an effective and safe treatment for pediatric patients with refractory BD.

P.C2.02.08

Therapeutic potential of immunosuppressive A151 ODN loaded liposomes in bleomycin induced mouse scleroderma model

***G. Kilic**, O. Bulut, M. Yildirim, G. Gucluler, T. Kahraman, B. Bayyurt Kocabas, I. Gursel;*
Bilkent University, Ankara, Turkey.

Introduction: Scleroderma (SSc) is an autoimmune disease which is characterized by vascular abnormalities, inflammation and fibrosis due to accumulation of extracellular matrix proteins. Although there are treatments for organ-specific complications of scleroderma, little is known concerning the therapy options related to resolution of ongoing fibrosis. In this study we investigated the immunosuppressive and anti-fibrotic effects of TLR antagonist A151 ODN in bleomycin induced fibrosis model.
Methods: NIH3T3 cells were stimulated with bleomycin and A151; fibrotic gene levels were assessed with qPCR. Inflammasome activation of mouse bone marrow derived macrophages upon p(dA/dT) stimulation in the absence or presence of A151 ODN was monitored via IL-1β levels of the culture supernatants. Lastly, to investigate whether A151 has a preventive effect on the development of scleroderma, mice were injected with A151 in free and liposomal forms 3 days before intratracheal bleomycin administration.
Results: Data revealed that TGFβ gene expression levels along with IL-1β production and expression of CD80 and CD86 from NIH3T3 and BMDMs were reduced in response to A151 treatments. A151 was able to decrease Col1a1 and Col1a2 gene levels from lung tissues as well as IL-6 and IL-12 production from BALF in mouse model of scleroderma.
Conclusion: These data indicated the preventive effect of A151 ODN on inflammation and fibrosis *in vitro* and in the mouse model of scleroderma. Current work focuses on demonstrating as a therapeutic value of A151 in established fibrosis.

P.C2.02.09

IL17- and IL22-producing γδ-T cells in the pathogenesis of systemic juvenile idiopathic arthritis

***B. Malengier-Devlies**[1], H. Engels[1], M. Imbrechts[1], J. Vandenhaute[1], T. Mitera[1], N. Berghmans[1], O. Burton[2], C. Wouters[3], P. Matthys[1];*
[1]Rega Institute, Laboratory of Immunobiology, Leuven, Belgium, [2]VIB-KU Leuven Center for Brain & Disease Research, Laboratory of Genetics of Autoimmunity, Leuven, Belgium, [3]University Hospitals Leuven, Leuven, Belgium.

Systemic juvenile idiopathic arthritis (sJIA) is a severe childhood immune disorder characterised by quotidian fever, rash, arthritis and splenomegaly and is associated with anaemia, neutrophilia and thrombocytosis. Although the aetiology of sJIA is poorly understood, the disease is considered as an autoinflammatory disorder driven by innate immune cell and cytokines. By using a novel mouse model for sJIA that rely on immunisation of Balb/c mice with complete Freund's adjuvant (CFA), we demonstrate in the present study a key role for innate γδ-T cells in the development of the disease. $CD27^{neg}$ γδ-T cells were dramatically increased in CFA-challenged mice and depletion of γδ-T cells ameliorated disease outcome. Intracellular cytokine staining identified γδ-T cells as a major source of IL-17 and IL-22, and neutralisation of each of these cytokines inhibited sJIA-like disease pathology. We further presented evidence for the involvement of γδ-T cells in the myelopoiesis of neutrophils and in the production of IL-1 and IL-6, and these are two key cytokines in the pathogenesis of sJIA for which their targets are currently used in the clinic. In conclusion, in a mouse model for sJIA, we provide evidence for a role of γδ-T cells in the aetiology and pathogenesis of this autoinflammatory disease. The data are clinically relevant as increased numbers of γδ-T cells were recently demonstrated in blood and joints of sJIA patients with active disease.

P.C2.02.10

Selective depletion of pro-inflammatory Th1 cells in chronic inflammation by targeting microRNA-148a with antagomirs

***P. Maschmeyer**[1], G. Petkau[1], F. Siracusa[1], J. Zimmermann[2], F. Zügel[1], A. A. Kühl[3], K. Lehmann[1], S. Schimmelpfennig[1], M. Weber[1], C. Haftmann[1], R. Riedel[4], M. Bardua[1], G. A. Heinz[1], C. L. Tran[1], B. F. Hoyer[5], F. Hiepe[3], S. Herzog[6], J. Wittmann[7], N. Rajewsky[8], F. Melchers[1], H. D. Chang[1], A. Radbruch[1], M. F. Mashreghi[1];*
[1]German Rheumatism Research Center (DRFZ) Berlin, Berlin, Germany, [2]Maurice Müller Laboratories (DKF), Bern, Switzerland, [3]Charité Universitätsmedizin Berlin, Berlin, Germany, [4]Max Planck Institute for Evolutionary Biology, Plön, Germany, [5]Universitätsklinikum Schleswig-Holstein, Kiel, Germany, [6]Medical University Innsbruck, Innsbruck, Austria, [7]Nikolaus-Fiebiger-Center, University of Erlangen-Nürnberg, Erlangen-Nürnberg, Germany, [8]Berlin Institute for Medical Systems Biology, Berlin, Germany.

CD4+ T helper (Th) cells contribute to the induction and maintenance of chronic-inflammatory diseases (CIDs). While depletion of Th cells with anti-CD4 antibodies significantly ameliorates disease activity in CID patients, this treatment also ablates Th cells that are protective against infections. Currently, a treatment that selectively targets CID-maintaining Th cells is still lacking.
The microRNA-148a (miR-148a) is expressed in CD4+ T helper type 1 (Th1) cells that reside at inflamed sites in patients with CIDs. Among Th cells, miR-148a expression is specifically upregulated in repeatedly-activated Th1 cells and promotes their survival by inhibiting the expression of the pro-apoptotic molecule Bim.
Here, we investigated whether miR-148a-targeting oligonucleotides (antagomir-148a) can be used to selectively deplete pro-inflammatory Th1 cells of chronic inflammation.
In the murine model of transfer colitis, antagomir-148a treatment reduced the number of Th1 cells in the colons of colitic mice by 50%. Moreover, antagomir-148a treatment inhibited miR-148a expression by 71% in colonic Th1 cells, while expression of the miR-148a target Bim was increased. Antagomir-148a-mediated reduction of Th1 cells resulted in a significant amelioration of colitis. The effect of antagomir-148a was selective for chronic inflammation. Antigen-specific memory Th cells that were generated by an acute immune reaction to nitrophenylacetyl-coupled chicken gamma globulin (NP-CGG) were not affected by treatment with antagomir-148a, both during the effector and the memory phase. In addition, antibody titers to NP-CGG were not altered. Thus, antagomir-148a might qualify as an effective drug to selectively deplete pro-inflammatory Th1 cells of chronic inflammation without affecting the protective immunological memory.

P.C2.02.11

The genetic regulation of the immune system in health and disease

V. Orrù[1], *M. Steri*[2], *C. Sidore*[2], *V. Serra*[1], *M. Marongiu*[1], *G. Sole*[2], *M. Pala*[2], *S. Lai*[1], *M. Dei*[1], *A. Mulas*[1], *M. Zoledziewska*[2], *M. Marongiu*[1], *M. Lobina*[1], *M. G. Piras*[1], *S. Olla*[2], *M. Floris*[3], *E. Fiorillo*[1], *F. Cucca*[2,3];
[1]*Institute for Genetic and Biomedical Research, Lanusei, Italy,* [2]*Institute for Genetic and Biomedical Research, Cagliari, Italy,* [3]*University of Sassari, Sassari, Italy.*

The immune system is a complex biological network of specialized cells and molecules that evolved to defend against pathogens and, in healthy condition, distinguishes between self and non-self antigens. Despite its relevance for human health, only a few studies have systematically evaluated the genetic influence on immune cells[1,2,3]. Some years ago, we completed the first GWAS assessing the genetic control of 95 leukocytes subsets in 2,870 general population individuals from the SardiNIA cohort. We identified multiple variant-trait associations at 13 loci, four of which overlapping with disease risk variants, revealing parameters potentially involved in disease pathogenesis[1]. The efficacy of this approach was demonstrated by our group that identified and clarified the mechanism of action of a complex variant, in the 3'UTR of the *BAFF* gene, associated with 18 immune endo-phenotypes and predisposing for multiple sclerosis and systemic lupus erythematosus[4]. Here we extended the previous characterization of immune cells to about 2,500 traits, related to the levels of 300 immune cell subtypes assessed in up to 4,000 volunteers from the SardiNIA cohort. We performed a GWAS, interrogating >26M variants, identifying 135 independent associations in 69 genetic loci (p-value<$6.9x10^{-11}$). Among these, 30 association signals are shared between immune traits and diseases. These data will help to understand the biological mechanisms underlying the links between pathologies and immune cells and to identify new therapeutic targets for personalized medicine. 1.Orrù V. *et al*, Cell 2013; 2.Roederer M. *et al*, Cell 2015; 3.Patin E. *et al*, Nat Immunol 2018; 4.Steri M. *et al*, NEJM 2017

P.C2.02.12

Effect of pregnancy hormones on CD4+ T cell activation and their possible use as treatment in multiple sclerosis

G. Papapavlou*, S. Hellberg, J. Raffetseder, J. Brynhildsen, M. Jenmalm, J. Ernerudh;*
Linköping Univesrity, Linköping, Sweden.

Multiple sclerosis (MS) is an autoimmune inflammatory disorder of the CNS with variable patient response to treatment. Intriguingly, women with MS show a transient improvement during pregnancy. Although little is known on the exact underlying mechanisms, the observed amelioration is most likely a result of immunological and hormonal interactions. The pregnancy hormones progesterone and estrogen could be involved in the systemic immune modulation as their levels coincide with disease amelioration and aggravation during and soon after pregnancy, respectively. Since a dysregulated activation of peripheral CD4+ T helper cells is considered to be a key event in MS pathogenesis, we examined the direct *in vitro* effects of these pregnancy hormones on CD4+ T cell activation derived from female healthy donors. The cells were stimulated for 24h with anti-CD3 and anti-CD28 antibodies and incubated in the presence of different concentrations of the hormones. Flow cytometry assessed the level of activation based on the expression of CD69 and CD25. Our preliminary data suggests that the hormones exert opposite effects, with progesterone hampering CD4+ cell activation ($n=4$, $p < 0.01$) and estrogen increasing it ($n=3$, $p > 0.05$), both in a dose-dependent manner. Our findings so far suggest that progesterone could be considered as a potent candidate of add-on treatments in MS, which so far has failed in the case of estrogen.

P.C2.02.13

Wiskott-Aldrich syndrome protein regulates endosomal architecture & TLRs signalling in DCs

G. M. Piperno[1]*, A. Naseem*[1]*, G. Silvestrelli*[1]*, N. Caronni*[1]*, K. E. Cervantes-Luevano*[1]*, N. Liv*[2]*, J. Klumperman*[2]*, H. Ali*[1]*, F. Graziano*[3]*, P. Benaroch*[3]*, H. Haecker*[4]*, R. Hanna*[5]*, F. Benvenuti*[1]*;*
[1]*International Centre for Genetic Engineering and Biotechnology, Trieste, Italy,* [2]*Section Cell Biology, Center for Molecular Medicine, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands,* [3]*Institute Curie Laboratoire Immunité et Cancer – INSERM U932 Transport Intracellulaire et Immunité, Paris, France,* [4]*Department of Infectious Diseases, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Menphis, United States,* [5]*Respiratory, Inflammation and Autoimmunity, MedImmune LLC, Gaithersburg, United States.*

Wiskott-Aldrich syndrome (WAS) is a rare immune deficiency caused by mutations in WAS protein (WASp), an actin nucleation promoting factor (NPFs) of the WASp/WAVE family, expressed exclusively in cells of the hematopoietic system. WAS mutated innate cells secrete excessive amount of cytokines in response to Toll-like receptor stimulation, contributing to autoimmune manifestations that often develop in WAS. However, the precise cellular mechanism of Toll-like receptor regulation by WASp remains elusive. Here we found that WASp nucleates actin around endosomes and promotes sorting and maturation of endolysosomes into degradative compartments in dendritic cells. Lack of WASp causes stalling of TLR9 and its ligands in hybrid, maturation-defective compartments, preventing degradation of signalling complexes. Delayed receptor and cargo degradation lower the threshold for receptor activation rendering DCs sensitive to little concentration of TLR9 agonists. These data elucidate how WASp negatively regulates endosomal TLRs signalling, contributing to explain disease pathogenesis.

P.C2.02.14

The regulation of IL-17 receptor signaling by kinases TBK1 and IKKε

D. Polatova*, H. Draberova, S. Janusova, K. Ruppova, A. Drobek, O. Stepanek, P. Draber;*
Institute of Molecular Genetics, Prague, Czech Republic.

Inflammation is an important reaction of the immune system in response to the infection and to the disruption of integrity of the organism. It is a way the organism immediately reacts to the damage or pathogen presence. Amongst important mediators of immune inflammatory response are members of interleukin 17 (IL17) family. IL-17 is a pivotal regulator of inflammatory immune reaction. Binding of IL-17 to its specific receptor leads to the production of pro-inflammatory cytokines such as IL-6, CXCL1, CXCL2, CCL20 or CCL2 which then promote an immune defense of the organism by recruitment of macrophages and neutrophils to the site of inflammation. The absence of IL-17 signaling leads to increased sensitivity to some pathogens (e.g. *Candida albicans*). On the other hand, the dysregulation of this signaling pathway results into severe autoimmune disorders such as psoriasis, rheumatoid arthritis or multiple sclerosis. Currently, antibodies blocking IL-17 or its receptor are approved for the treatment of psoriasis and psoriatic arthritis. As clinical therapy by antibodies has numerous limitations and brings many side effects, the elucidation of the precise mechanism of yet not completely understood signaling via IL-17 receptor is a possible way to reveal new potential drug targets for therapeutic treatment. Here we aimed to understand the role of kinases TBK1 or IKKε in propagating IL17 receptor-triggered signaling pathways and to elucidate whether the inhibition or ablation of these kinases can be used to modulate the IL17-induced cellular responses.

P.C2.02.15

Imbalance of blood Tfh and Tfr cells in systemic and organ-specific human autoimmunity

F. Ribeiro[1,2]*, V. R. Fonseca*[1,3]*, A. Agua-Doce*[1,2]*, V. Romao*[1,3]*, E. Nobre*[3]*, M. J. Bugalho*[3]*, J. E. Fonseca*[1,3]*, L. Graca*[1,2]*;*
[1]*Instituto de Medicina Molecular João Lobo Antunes, Lisboa, Portugal,* [2]*Instituto Gulbenkian da Ciência, Lisboa, Portugal,* [3]*Centro Hospitalar Lisboa Norte – Hospital de Santa Maria, Lisboa, Portugal.*

Many autoimmune diseases are mediated by self-reactive antibodies produced during disturbed B-T cell interactions in the germinal center (GC). The balance between T follicular helper (Tfh) cells and T follicular regulatory (Tfr) cells have a significant impact on GC outcome: while Tfh cells support the production of high-affinity antibodies, Tfr cells limit the production of self-reactive antibodies. We have recently found that the balance between circulating Tfh and Tfr cells is altered in human systemic autoimmunity. In order to investigate whether such dysregulation is also observed in organ-specific autoimmunity, we compared Sjögren Syndrome (SS) and Hashimoto's Thyroiditis (HT) patients.
We analyzed peripheral blood Tfh and Tfr cells of those two distinct autoantibody-mediated autoimmune diseases. We found a significant increase in Tfr/Tfh ratio in peripheral blood of SS patients compared to age-matched healthy donors, contrary to HT patients where a considerable decrease in Tfr/Tfh ratio was observed. However, circulating Tfh cells in both diseases expressed GC-related activation markers, suggesting greater Tfh cell activation in autoimmune patients regardless of the underlying disease.
Our results show that SS and HT are characterized by dysregulation of Tfh/Tfr ratio, possibly implicated in the loss of self-tolerance and emergence of autoantibodies. However, the imbalance of circulating Tfr and Tfh cells is distinct in these diseases, suggesting they do not share common mechanisms of GC dysregulation. Our results suggest that the blood Tfr/Tfh ratio may constitute a novel biomarker for autoantibody-mediated autoimmunity, potentially identifying patients with greater benefit for therapeutic approaches targeting B-T cell interactions.

P.C2.02.16

Pro-survival phenotype of human endothelial cells exposed to neutrophil-derived extracellular vesicles

M. Surmiak[1]*, S. Polanski*[2]*, J. Kosalka*[1]*, M. Sanak*[1]*;*
[1]*Department of Internal Medicine, Jagiellonian University Medical College, Krakow, Poland,* [2]*University Hospital, Krakow, Poland.*

Introduction In autoimmune vasculitis syndromes like granulomatosis with polyangiitis (GPA) neutrophils can damage multiple organs. In this study we measured transcriptional response of human endothelial cells to extracellular vesicles from neutrophils activated with IgG anti-PR3 antibodies. **Material and methods** Anti-PR3 antibodies were isolated form serum of patients with GPA by affinity chromatography. Neutrophils from healthy volunteers were primed with TNF-α (2ng/mL) and stimulated with anti-PR3 (200 ng/mL) for 2h. Extracellular vesicles (EV) were collected from culture by ultracentrifugation, characterized by flow cytometry (CD9, CD63, CD81) and nanoparticle tracking analysis. After 6 hours stimulation of human umbilical vein endothelial cells (HUVEC) by EV, RNA was isolated, reverse transcribed and measured by real-time quantitative PCR method (TaqMan Inflammation and Apoptosis Panels).

Results Transcripts were compared between primed only and EV stimulated HUVEC. Significant up-regulation (>2 fold change, p<0.05) was present for 8 transcripts. Some were inhibitors of apoptosis (*BIRC1*, *BIRC2*) and NF-κB modulators (*CARD6* and *NALP*), others regulated vascular adherence or leak (*VCAM1*, *PTGIS*, *KLK14, IL1R2*). Significant down-regulation was consistent with apoptosis inhibition (*CASP8, HRK, BCL2)* and also present for 2 other transcripts (*HTR3A, KLKB1)*. **Conclusions** This in vitro model of neutrophil-derived EV influence on endothelium in autoimmune vasculitis revealed unexpected changes in genes expression. Pro-survival phenotype was suggested by altered transcripts, accompanied by increase of prostacyclin synthase and shift in kallikreins. We conclude, that observed reprogramming of endothelial phenotype can enhance granulocyte transmigration and maintain microvascular perfusion in damaged tissue. Supported by National Center of Science in Poland, grant number: 2016/21/D/NZ6/02123

P.C2.02.17
the kinase MAP4K3/GLK is a novel therapeutic target for IL-17A-mediated autoimmune diseases

*H. Chuang, **T. Tan**;*
Natioanl Health Research Institutes, Zhunan, Taiwan.

T-cell receptor signaling activates the kinase MAP4K3 (also named GLK) by inducing its direct interaction with the upstream adaptor protein SLP-76. Activated GLK directly phosphorylates and activates PKC-θ, which is required for NF-κB activation in T cells. Moreover, GLK-deficient mice show impaired Th17 differentiation and are resistant to IL-17A-mediated experimental autoimmune encephalomyelitis (EAE). Consistently, autoimmune SLE and rheumatoid arthritis (RA) patients show significantly increased GLK levels in T cells; the percentage of GLK-overexpressing T cells is correlated with autoimmune disease severity.
Recently, we generated and characterized T-cell specific GLK transgenic mice and found that these transgenic mice spontaneously developed autoimmune diseases with an induction of systemic inflammation and an increase of autoantibodies (ANA, anti-dsDNA, rheumatoid factor). We found that GLK signaling specifically induced IL-17A transcription in the T cells of GLK transgenic mice. GLK-mediated IL-17A induction has been studied using biochemical approaches, genetically modified mice, and autoimmune patient T cells. We will present the data on a novel signal transduction mechanism of IL-17A transcriptional activation by GLK in autoimmune T cells and activated T cells. Collectively, MAP4K3/GLK is a diagnostic biomarker and therapeutic target for IL-17A-mediated autoimmune diseases.

P.C2.02.18
***Ex-vivo* beneficial effect of interferon-β treatment on the secretion profile of inflammatory mediators via suppression of iNOS signaling pathway in patients with primary Sjögren's syndrome**

*S. Benchabane[1], M. Belkhelfa[1], H. Belguendouz[1], S. Zidi[1], A. Boudjelida[2], **C. Touil-Boukoffa**[1];*
[1]Cytokines and NO Synthases Group. Faculty of Biological Sciences,, Algiers, Algeria, [2]Internal medicine department, Maillot Hospital, Algiers, Algeria.

Introduction Primary Sjögren's syndrome (pSS) represents a chronic, systemic autoimmune disorder, characterized by lymphocytic infiltration of exocrine glands. Increasing evidence had revealed that inflammatory mediators, such as nitric oxide (NO) and pro-inflammatory cytokines are critical in the development and perpetuation of pSS. In our study, we investigate the *ex vivo* immunomodulatory effect of interferon-β on iNOS expression, as well as on pro-inflammatory (tumor necrosis factor (TNF)-α, interleukin (IL)-6) and immunoregulatory (IL-10) cytokines production. Furthermore, we examined potential associations between the influence of IFN-β treatment on NO production and pSS clinical and serological manifestations. ***Methods*** In 41 pSS patients documented for their clinical and serological features, NO and cytokines levels were measured by the Griess method and enzyme-linked immunosorbent assay, respectively.
Inducible nitric oxide synthase expression was analyzed by fluorescence immunostaining assay, using peripheral blood mononuclear cells (PBMCs) isolated from healthy controls and pSS patients. ***Results*** Our results revealed a strong down-modulating effect of IFN-β in the secretion of pro-inflammatory mediators including TNF-α, IL-6, and NO production. Interestingly, IFN-β exerts an increase in IL-10 levels. The most suppressive effect exerted by IFN-β on NO production was importantly reported for patients with neurological manifestation. This immunomodulatory effect of IFN-β on NO production is highly related to the decrease of inducible nitric oxide synthase (iNOS) expression. ***Conclusion*** Our findings highlight a consistent *ex vivo* inhibitory effect of IFN-β on pro-inflammatory cytokines production and NO pathway in pSS patients. Our data suggest that IFN-β could represent a potential candidate for targeting inflammation during pSS.

P.C2.02.19
Investigating genetic variation in the control of human T cell activation

***C. Williams**, T. Hou, L. Faulkner, D. Sansom;*
Institute of Immunity and Transplantation, London, United Kingdom.

Despite the identification of vast numbers of genetic loci implicated in autoimmune susceptibility achieved through GWAS, interpretation of the functional consequence of these loci is complicated by; the polygenetic nature of complex autoimmunity, the haplotype structure of SNPs in linkage disequilibrium and the high frequency of causal variants in non-coding regions of DNA. Enrichment of risk loci has been observed in T cell enhancers, specifically those associated with stimulation. This implies T cell activation as an integral element of autoimmunity through which functional aberration as a result of genetic variation may be observed. The two signal model of T cell activation describes the response of a T cell as a function of the dose of two signals mediated through TCR and CD28. Thus, fine tuning of T cell stimulation may expose functional diversity between individuals bearing different SNPs. The polygenetic nature of autoimmunity suggests that multiple risk variants integrate into functional pathways. As strongly deleterious mutations would likely be selected against, these variants likely also confer subtle (non-disease-causing) effects in T cell responses in healthy individuals. We have set up defined T cell stimulation assays in an attempt to identify functionally relevant SNPs through analysis of inter-individual variation in T cell responses from healthy donors. These assays have demonstrated a considerable degree of variability regarding T cell phenotype and proliferative response thereby indicating differential sensitivity to TCR and CD28 co-stimulation. Combining genetic and functional analysis will allow us to identify SNPs associated with specific T cell outcomes to stimulation.

P.C2.02.20
Microglia are myelinogenic and neuroprotective macrophages of the CNS

***A. Wlodarczyk**, A. Benmamar--Badel, K. Nolling Jensen, T. Owens;*
Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark.

Microglia are central nervous system (CNS)-resident macrophages. They are implicated in neuroinflammatory and neurodegenerative diseases including multiple sclerosis. We have shown that numbers of microglia expressing CD11c, normally almost undetectable in adult CNS, significantly increase in experimental autoimmune encephalomyelitis (EAE). These are effective antigen presenting cells, but poor inducers of Th1 or Th17 responses. Interestingly, CD11c$^+$ microglia express high levels of neuroprotective insulin-like growth factor 1 (IGF1), suggesting a neuroprotective rather than proinflammatory role. We have recently shown that CD11c+ microglia cells predominate in primary myelinating areas of the developing brain and express genes for neuronal and glial survival, migration and differentiation, and they control primary myelination via IGF1 production.
Here we show that upon adoptive transfer into the cerebrospinal fluid of adult mice with symptomatic EAE, neonatal microglia migrate to the inflammatory lesions in the spinal cord. This intervention suppressed disease symptoms and reduced leukocyte infiltration and demyelination. Although unfractionated neonatal microglia suppressed disease, the CD11c+ microglial subset was significantly most effective. We therefore identify a unique phenotype of neonatal microglia that have re-myelinating and anti-inflammatory potential. Understanding mechanism for these protective effects will enable therapy for neuroinflammatory disease.

P.C2.03 Immune signaling and therapy in autoimmunity - Part 3

P.C2.03.01
Combined actions of Type 1 diabetes associated HLA genes, alleles and residues

***A. Alansari**, S. Al-Badi, M. Al-Balushi, H. Al-Riyami, S. Al-Yaarubi;*
Sultan Qaboos University, Al-Khoud, Oman.

Introduction. Genetic susceptibility and environmental factors determine the onset of type 1 diabetes (T1D). The identification of human leukocytes antigen (HLA) high risk alleles, genotypes and haplotypes is beneficial for understanding their roles in T1D pathogenesis and intervention practices. Also, it could help in developing new assays and immunotherapy. Aim. The aim of the study is identify associations between HLA genes and T1D in Oman. Materials and methods. Our case-control study included 100 diabetic children (mean age 9.19±3.94 years) and 110 controls (mean age 10.77±3.36 years). HLA-DRB1, DQA1 and DQB1 alleles were genotyped using sequence specific primer polymerase chain reaction (SSP-PCR). Results. B*08, DQB1*02, DRB1*03 and DRB1*04 alleles were associated with T1D susceptibility, while C*16, B*51, DQB1*05 and DQB1*06 alleles were associated with protection. HLA- DRB1*03 and DQB1*02 alleles showed the strongest risk association among all alleles. Six DRB1 residues (Glu-9, Ser-11, Ser-13, Tyr-30, Val-70, Lys-71) were significantly associated with TID susceptibility and analysis indicated that they have combined actions. Heterozygous genotypes, HLA-DRB1*03/*04 and DQB1*02/*03 were associated with T1D susceptibility significantly (p=0.021, OR=15.909). Furthermore, HLA-DRB1*03 and DQ*02 found to be in LD and have significant combined action in the disease (p=5.21E-13, OR 12.11). Conclusions. Significant association of HLA I and II alleles, genotypes and haplotypes were identified in Omani TID patients. Similar associations were reported from Arab and non-Arab populations. Results indicated significant combined actions predisposing to TID at the genes, alleles and resides levels.

P.C2.03.02

Annexin A1 (ANXA1): potential regulator of adaptive immune response in Multiple Sclerosis (MS)

A. Colamatteo[1], *E. Maggioli*[2], *G. Cali*[3], *S. Cassano*[3], *M. Galgani*[3], *D. Bruzzese*[4], *G. Maniscalco*[5], *G. Matarese*[1], *E. Solito*[2], *V. De Rosa*[3];
[1]*Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Napoli, Italy,* [2]*William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom,* [3]*Istituto di Endocrinologia ed Oncologia Sperimentale, Consiglio Nazionale delle Ricerche, Napoli, Italy,* [4]*Dipartimento di Sanità Pubblica, Università degli Studi di Napoli "Federico II", Napoli, Italy,* [5]*Dipartimento di Neurologia, Centro Regionale Sclerosi Multipla, Azienda Ospedaliera "A. Cardarelli", Napoli, Italy.*

ANXA1 is a glucocorticoid-regulated anti-inflammatory protein, which acts as an endogenous regulator of innate immunity by limiting neutrophils extravasation and blocking monocytes migration. ANXA1 is also expressed in brain microvascular endothelial cells, where it regulates blood brain barrier (BBB) integrity. Recently, a selective loss of ANXA1 in the plasma and cerebrovascular endothelium of multiple sclerosis (MS) subjects has been reported.
Our aim was to investigate whether ANXA1 could have also a role in the modulation of immune tolerance in naive-to-treatment relapsing-remitting (RR)-MS subjects. We analysed whether its plasma levels correlated with disease severity and progression. Moreover, we measured ANXA1 expression in different T cell subsets: regulatory T (Treg), conventional T (Tconv) and Th17 cells. We also investigated the migratory capacity of Treg and Tconv cells from RRMS and healthy subjects. Finally, we assessed whether recombinant (r)-human ANXA1 treatment could affect T cell activation, proliferation and metabolic engagement *in vitro*.
We observed that ANXA1 plasma levels inversely correlated with EDSS and relapses number in RRMS subjects. Its expression was significantly lower in Treg, Tconv and Th17 cells from RRMS compared to healthy subjects. This finding correlated with a higher degree of adhesion and migration of both Treg and Tconv cells *in vitro*. Rh-ANXA1 treatment profoundly reduced proliferation, expression of several activation markers and glycolytic engagement of PBMCs from healthy subjects, with a mild effect in RRMS subjects. Understanding the molecular mechanism accounting for the reduced ANXA1 expression should provide relevant informations on the key events leading to RRMS onset and progression.

P.C2.03.03

Dimethyl fumarate induces an anti-inflammatory shift in follicular T cells in multiple sclerosis

V. Cunill[1,2], *M. Massot*[1], *A. Clemente*[2], *C. Callles*[1], *V. Andreu*[2], *C. Capó-Serrra*[2], *V. Núñez*[1], *A. López-Gómez*[1,2], *R. Díaz*[1], *M. Jiménez*[1], *J. Pons*[1,2], *C. Vives*[2], *J. Ferrer*[1,2];
[1]*Hospital Universitari Son Espases, Palma de Mallorca, Spain,* [2]*Fundació Institut d'Investigació Sanitària Illes Balears, Palma, Spain.*

Introduction: Although Multiple sclerosis (MS) is considered a T cell-mediated autoimmune disease, several evidences demonstrate the involvement of B cells in its etiology. Follicular helper (Tfh) cells are essential in the regulation of humoral immunity. Alterations in circulating(c)Tfh function and/or distribution associate with autoimmune diseases including MS. Dimethyl fumarate (DMF) is a recently approved first-line treatment for relapsing-remitting MS(RRMS) patients. The aim of our study was to evaluate cTfh subpopulations in RRMS patients, the impact of DMF treatment on cTfh distribution and their clinical evolution. **Methods:** We analyzed, by flow cytometry, the distribution of cTfh1(CXCR3+CCR6-), cTfh2(CXCR3-CCR6-), cTfh17(CXCR3-CCR6+) and cTfh17.1(CXCR3+CCR6+) in CD4+ follicular T cells (CD45RA-CXCR5+) subpopulations from 29 untreated RRMS patients and 6 and 12 months after DMF treatment. We also evaluated these subpopulations in CD4+ non-follicular T cells (CD45RA-CXCR5-). **Results:** Untreated RRMS patients presented higher percentages of cTfh17.1 cells and lower percentages of cTfh2 cells consistent with a pro-inflammatory bias compared to controls. DMF treatment induced a progressive increase in cTfh2 cells 6 and 12 months after treatment, accompanied by a decrease in cTfh1 and the pathogenic cTfh17.1 cells. Similar decreases of non-follicular Th1 and Th17.1 cells in addition to an increase in the anti-inflammatory Th2 subpopulation were also detected. Interestingly, we identified three non-responders DMF patients unable to normalize the deviation of cTfh subpopulations. **Conclusions:** RRMS have a pro-inflammatory profile, defined by high cTfh17.1 and low cTfh2 subpopulations, which can revert after DMF treatment. Monitoring cTfh subsets during treatment may become a biological marker of DMF effectiveness.

P.C2.03.04

Reciprocal modulation of human Th1 and Th17 cells by beta2-adrenergic receptor agonists

P. J. Darlington[1], *M. Tabatabaei Shafiei*[2], *T. Daigneault*[1], *C. M. Carvajal Gonczi*[1];
[1]*Concordia University, Montreal, Canada,* [2]*McGill University, Montreal, Canada.*

Introduction: T helper (Th) 1 and Th17 cells are essential for host protection and have been linked to various autoimmune diseases. The beta2-adrenergic receptor (β2AR) pathway regulates physiological reactions in response to catecholamines hormones such as epinephrine and norepinephrine. Little is known about how catecholamines alter Th cell balance in health and disease in humans. **Methods:** Peripheral blood mononuclear cells (PBMC) from healthy human donors, and Th cells purified with magnetic columns, were activated *in vitro* with anti-CD3 and anti-CD28, and treated with β2AR pharmacological agonists (terbutaline, or nebivolol) and antagonist (ICI-118,551). Proliferation was measured by flow cytometry and cytokines were measured by enzyme linked immunosorbent assay. Common polymorphisms of β2AR (*ADRB2*) were determined by Sanger sequencing. **Results:** Th17 cells expressed β2AR. Terbutaline raised the level of Th17 cell cytokine IL-17A ($p<0.01$) while lowering the level of Th1 cell cytokine IFNγ ($p<0.01$). Nebivolol, an inverse agonist, lowered IL-17A but raised IFNγ which was opposite to the terbutaline effect. Only a slight proliferative change was observed on Th cells ($p<0.001$). The cAMP analog (dbcAMP) raised IL-17A ($p<0.05$) while lowering IFNγ ($p<0.001$) similar to the terbutaline effect. Samples from donors who were *ADRB2* Arg16 heterozygotes responded significantly to terbutaline ($p<0.01$) while Gln/Glu27 had no interaction with agonist response. **Conclusion:** Cell signaling through β2AR alters the balance of Th17 and Th1 cytokines and may be linked to common polymorphisms. These results suggest that β2AR agonists differentially modulate adaptive immune responses and may influence autoimmunity. Research funded by NSERC Discovery grant (418522-2013)

P.C2.03.05

Identification and characterization of HDAC1 interaction networks in Th17 cells

P. Hamminger[1], *L. Hess*[2], *L. Göschl*[1], *T. Preglej*[1], *M. Hartl*[3], *C. Seiser*[2], *W. Ellmeier*[1];
[1]*Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria,* [2]*Center for Anatomy and Cell Biology, Division of Cell and Developmental Biology, Vienna, Austria,* [3]*Mass Spectrometry Facility, MFPL, Vienna, Austria.*

The differentiation and function of $CD4^+$ T helper (Th) subsets has to be tightly regulated, since their dysregulation is linked with immune-mediated diseases. Th cell differentiation is accompanied by reversible changes in histone acetylation, mediated by the opposing activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs), however many non-histone targets are emerging, indicating that HAT/HDACs act beyond the regulation of chromatin. Results of my laboratory demonstrate an essential role for HDAC1 in regulating Th17 cell effector function and that loss of HDAC1 in T cells protects mice from the development of experimental autoimmune encephalitis. This clearly indicates essential roles for HDAC1 in the control of T cell-mediated autoimmunity. Since HDAC1 is part of larger multiprotein complexes, it is tempting to speculate that the crucial role of HDAC1 is mediated by targeting factors that are key regulators of Th17 cells. The aim of my PhD thesis project is to test this hypothesis.

P.C2.03.06

In-depth mapping of transcriptomic dysregulation in circulating pDCs from patients with Sjögren's Syndrome associated with increased production of pro-inflammatory cytokines

M. R. Hillen, *A. Pandit, S. L. Blokland, S. A. Hartgring, K. van der Wurff-Jacobs, A. A. Kruize, M. Rossato, J. A. van Roon, T. R. Radstake;
University Medical Center Utrecht, Utrecht, Netherlands.*

Introduction: Primary Sjögren's syndrome (pSS) is an autoimmune disease characterized by lymphocytic infiltration of the exocrine glands. Type-I interferons (IFN) are thought to play an important role in pSS pathogenesis, suggesting a role for pDCs. We performed RNA sequencing on isolated circulating pDCs from patients with pSS, non-Sjögren's sicca (nSS) patients, and healthy controls (HC).
Methods: Peripheral blood pDCs were isolated from two independent cohorts (each n=31) of patients and controls using MACS and RNA sequencing was performed. ±20 million paired-end sequencing reads per sample were obtained using using Illumina HiSeq 2500 platform. For *in vitro* experiments, pDCs were isolated from 22 pSS and 17 HCs and cultured for 3h with loxoribine or CPG-C.
Results: 3144 genes were consistently differentially expressed (p-value <0.05) between all groups in both cohorts. We generated gene modules from both cohorts and found 5 gene clusters that were consistently dysregulated. Pathway analysis showed that these clusters contain genes associated with cellular activation, including IFN-signalling and viral sensing. pDCs from pSS patients produced significantly more IFN-a and IFN-b *in vitro* and cytokine levels correlated with the expression of genes that were present in the RNAseq gene clusters.
Conclusions: We identified gene clusters that are robustly replicated in two independent cohorts. nSS patients showed similar transcriptomic dysregulation to pSS patients at an intermediate level. The increased production of type-I IFN by pSS pDCs confirms their activated phenotype and the association between cytokine production and gene expression suggests that these transcriptomic difference may underpin pDC dysregulation in pSS.

P.C2.03.07
Effects of ROS-insensitive PTPN22 on autoimmunity

***J. James**, C. Marquina, A. Saxena, R. Holmdahl;*
Karolinska Institute, Solna, Sweden.

Autoimmune diseases present an ever-growing health concern and affect millions around the globe, particularly in Western countries. An allelic variant of PTPN22 (protein tyrosine phosphatase, non-receptor type 22) is highly associated with several autoimmune diseases such as rheumatoid arthritis, SLE and type 1 diabetes. PTPN22 acts a negative regulator of signalling in B and T cells by dephosphorylating immunoreceptor-proximal proteins. Furthermore, PTPs act as redox sensors and inactivation of PTPs via oxidation plays an important role as a regulatory mechanism. While studies have focused on the disease-associated PTPN22 R620W variant, the oxidative regulation of PTPN22 in autoimmunity has not been investigated yet.
To this purpose our lab has developed a mouse strain where a point mutation (C129S) in the PTPN22 gene results in insensitivity to redox regulation. Preliminary results show similar PTPN22 expression levels in splenic B cells and thymocytes and comparable immune cell profiles in spleen, thymus and bone marrow between wild-type and PTPN22^{C129S} mice. Phosphorylation of target proteins downstream of PTPN22 is unaffected by the mutation. However, GPI-induced arthritis and DTH (delayed-type hypersensitivity) models show increased arthritis severity in the PTPN22 mutant. How PTPN22 function is regulated by ROS remains to be further investigated.

P.C2.03.08
The properties of integrin α4β7+ CD4 T cells are altered in multiple sclerosis

***M. Nguyen Ky**[1], A. Ruet[2], A. Bru[1], C. Dulau[2], M. Deloire[2], K. Kounkou[2], J. Déchanet-Merville[1], P. Blanco[1], B. Brochet[2], N. Schmitt[1];*
[1]ImmunoConcEpT CNRS UMR 5164, University of Bordeaux, Bordeaux, France, [2]Neurology department, CHU Bordeaux Hospital, Bordeaux, France.

Multiple sclerosis (MS) is a neurodegenerative autoimmune disease of the central nervous system (CNS). Mouse studies suggest that gut-derived CD4 T cells might be an important player in MS pathogenesis. CD4 T cells primed in the gut are characterized by their expression of integrin α4β7. α4β7+ CD4 T are negative for the expression of the brain-homing molecule integrin α4β1 but express the other main brain homing molecule LFA-1 proposed to play an important role in the migration of CD4 T cells such as Th17 into CNS in mouse models.
We found that the proportion of Th17 cells was significantly increased in α4β7+ CD4 T cells in the blood of MS patients compared to controls. Following polyclonal stimulation, we observed an increased proportion of IL-2 and a decreased proportion of IFNγ and MIP-1α-secreting cells in α4β7+ CD4 T cells in MS compared to controls while IL-17 and IL-10 levels were not altered. Importantly, these modifications were more marked in α4β7+ CD4 T cells compared to α4β7- CD4 T cells.
We next assessed whether the capacity of α4β7+ CD4 T cells to migrate into the CNS might be altered by Natalizumab (NZB), a monoclonal antibody targeting integrin α4, which efficiently prevents the migration of pathogenic CD4 T cells into the CNS. We found that NZB indirectly decreased the expression of LFA-1 on α4β7+ CD4 T cells suggesting that NZB treatment might reduce their migration to the CNS.
Altogether, these results suggest the involvement of α4β7+ CD4 T cells in MS pathogenesis.

P.C2.03.10
***KIR* genes and autoantibodies levels in rheumatoid arthritis**

***I. V. Reyes-Pérez**[1], P. E. Sánchez-Hernández[1], J. F. Muñoz-Valle[2], G. E. Martínez-Bonilla[3], S. Gutiérrez-Ureña[3], S. Cerpa-Cruz[3], V. González-Díaz[3], T. García-Iglesias[1], J. Polanco-Cruz[3], K. M. García-Osuna[3], E. E. Velarde-De la Cruz[1], M. G. Ramírez-Dueñas[1];*
[1]Laboratorio de Inmunología, Universidad de Guadalajara, Guadalajara, Mexico, [2]Instituto de Investigación en Ciencias Biomédicas, Universidad de Guadalajara, Guadalajara, Mexico, [3]Servicio de Reumatología, Hospital Civil Fray Antonio Alcalde, Guadalajara, Mexico.

Introduction Rheumatoid arthritis (RA) is an autoimmune disease characterized by inflammation of the diarthrodial joints and production of different autoantibodies such as RF, anti-CCP, anti-MCV and anti-PADI4. *KIR* genes encode receptors that regulate the function of NK cells and T cell subpopulations, which may be involved in the activation of B cells. **Aim** To identify the association of *KIR* genes with RF, anti-CCP, anti-MCV and anti-PADI4 levels in rheumatoid arthritis **Methodology** Peripheral blood samples were obtained from RA patients (RA, n=90) and healthy subjects (HS, n=70). gDNA was extracted by Miller modified technique and 16 KIR genes were typed by PCR-SSP. RF was quantified by turbidimetry. Anti-CCP, anti-MCV, and Anti-PADI4 were quantified by ELISA kit. The data were analyzed with chi-square and t-student tests with a significant p <0.05. **Results** *KIR2DL2* and *KIR2DS4del* were found more frequently in patients than in healthy subjects (p = <0.0001; p = <0.003, respectively). RF (HS=6.7 U/mL, AR=62.3 U/mL, p=0,0001), anti-CCP (HS=0.6 U/mL, AR=150.6 U/mL, p=0,0001), anti-MCV (HS=10.1 U/ml, AR=1418.0, p=0.0001) and anti-PADI4 (HS=2.57 ng/mL, AR=4.04 ng/mL, p=0.0001) were higher in subjects with RA than in HS. *KIR2DL2- / 2DS4del+* genotype obtained a tendency to increase anti-PADI4 levels in comparison to *KIR2DL2+/2DS4del+ (p*=0.07). **Conclusions** *KIR2DL2* and *KIR2DS4del* could act as risk factors in the AR development. An association between *KIR* genes and autoantibodies levels has not been found yet, but the data shows a tendency of higher levels of anti-PADI4 in patients with *KIR2DL2+/2DS4del+* genotype.

P.C2.03.11
Altered Toll-like receptor -7 and -9 signaling in patients with primary Sjögren's syndrome: a single-cell network analysis by mass cytometry

***I. Sarkar**[1], R. Davies[1], D. Hammenfors[1,2], B. Bergum[1], A. K. Aarebrot[1], S. M. Solberg[1,3], P. Vogelsang[1], J. G. Brun[2,4], R. Jonsson[1], S. Appel[1];*
[1]Broegelmann Research Laboratory, Department of Clinical Science, University of Bergen, Bergen, Norway, [2]Department of Rheumatology, Haukeland University Hospital, Bergen, Norway, [3]Department of Dermatology, Haukeland University Hospital, Bergen, Norway, [4]Department of Clinical Science, University of Bergen, Bergen, Norway, Bergen, Norway.

Introduction: Primary Sjögren's syndrome (pSS) is a chronic, inflammatory, systemic autoimmune disorder characterized by dysfunction of exocrine glands, mainly salivary and lacrimal, leading to dry mouth and eyes. The dryness and other clinical manifestations result in a significant decrease in life quality of the patients, 90 % of them being women. Currently there is no cure or effective treatment for pSS due to the pathogenic complexity of the disease, with genetic predisposition, hormonal and environmental factors all contributing to the disease etiology. Enhanced activation potential of signaling pathways may play a crucial role in the pathogenesis of pSS. In our previous work using phospho-flow cytometry, we had analysed MAPK/ERK and JAK/STAT signaling networks in peripheral blood mononuclear cells (PBMCs) and observed altered signaling profiles in pSS patients. Methods: Mass cytometry was used to validate and refine our previous results. Phosphorylation status of MAPK/ERK and JAK/STAT signaling networks in Toll-like receptor (TLR) -7 and -9 stimulated PBMCs from 25 female pSS patients and 25 age and sex-matched healthy donors were analysed. Results: Stimulation of PBMCs with TLR-7 and -9 ligands resulted in significant differences in the phosphorylation profiles between patients and healthy donors. Principal component analysis (PCA) including clinical parameters showed that it was possible to subgroup the patients. Conclusion: Patients with pSS have an increased signaling potential in PBMCs upon TLR-7 and -9 stimulation. Funding: This work was supported by the EU H2020 contract HarmonicSS (H2020-SC1-2016-RTD/731944), Broegelmann Foundation, Western Norway Regional Health Authorities (grant nr. 912065) and University of Bergen.

P.C2.03.12
Expanding systemic antigen specific regulatory networks to treat liver autoimmunity

***C. Sokke Umeshappa**, S. Singha, K. Shao, R. Hebbandi Nanjundappa, J. Lee, J. Yamanouchi1, Y. Yang, P. Santamaria;*
University of Calgary, Calgary, Canada.

Peptide-major histocompatibility complex class II (pMHCII)-based nanomedicines trigger the formation of multi-cellular regulatory networks by re-programming autoantigen-experienced CD4+ T-cells into tissue-specific T-regulatory type 1 cells (TR1). Here, we show that pMHCII-based nanomedicines displaying liver autoimmune disease-relevant, yet ubiquitous mitochondrial, endoplasmic reticulum or cytoplasmic, antigens can blunt multiple liver autoimmune diseases, Primary Biliary Cholangitis (PBC), Autoimmune Hepatitis (AIH), and Primary Sclerosing Cholangitis (PSC). The therapeutic effect occurred in an autoimmune-disease as well as non-disease-but-organ-specific manner, wherein PBC-relevant pMHCII nanomedicines blunted not only PBC but also AIH and PSC, and AIH-relevant pMHCII nanomedicines blunted not only AIH but also PBC and PSC. These data indicate that hepatocyte and biliary epithelial cell destruction in liver autoimmunity results in the activation of subdominant T-cells recognizing systemically-expressed antigens not known to partake in these diseases. Mechanistically, nanomedicines-expanded TR1 cells suppressed autoantigen-loaded antigen-presenting cells and drove cognate B cells differentiation into disease-suppressing B regulatory cells locally, without suppressing immunity against infections or cancer. From the clinical point, the therapeutic effect was superior to Ursodeoxycholic acid, the current standard of care for PBC. Thus, the current study represents an unmet clinical need for three complex liver autoimmune diseases.

P.C2.03.13

Changes in natural killer cells and monocytes during the third trimester of pregnancy in multiple sclerosis

***H. B. Søndergaard**, L. Börnsen, J. R. Christensen, B. R. Nielsen, A. Oturai, F. Sellebjerg;*
Danish Multiple Sclerosis Center, University of Copenhagen, Rigshospitalet, Copenhagen, Denmark.

Pregnancy affects the disease course in the autoimmune disease multiple sclerosis (MS), particularly in the third trimester, where the relapse rate is reduced by as much as two thirds. This study aimed at identifying specific changes in biomarkers of immune activation in pregnant MS patients. Flow cytometry analysis was performed on mononuclear blood cells stained with antibodies directed against, among others, PD-L1 and PD-L2, antigen-presenting cells (APCs), NK-cells, NKT-cells, CD4+- and CD8+ T-cells and subsets of these cell types. Pregnant MS patients had a highly significant increase in the percentage of monocytes and a decrease of NK-cells and myeloid dendritic cells (mDCs) compared to non-pregnant MS patients. No changes were found in NKT- and regulatory T-cells, CD4+- and CD8+-T-cells, plasmacytoid dendritic cells (pDCs) or B-cells. The percentage of mDCs and pDCs expressing PD-L1 was increased and the percentage of monocytes and pDCs expressing PD-L2 was increased in pregnant patients. PD-L1 and PD-L2 are known to bind PD-1 expressed on T cells with an inhibitory effect on T-cell proliferation and increase in the production interleukin-10. Also, we confirm previous reports of a relative increase in CD56-bright NK cells and a decrease in CD56-dim NK cells in the last trimester of pregnancy in MS patients. Thus, decreased immune activation in the third trimester of pregnancy coincides with an increased number of circulating monocytes and lower numbers of NK-cells together with a more immunoregulatory APC phenotype. This research was funded by the Danish Council for Strategic Research and the Danish Multiple Sclerosis Society.

P.C2.03.14

New insights into inflammatory myopathies using microarray data analysis

***C. Sordo-Bahamonde**, S. Lorenzo-Herrero, A. López-Soto, E. deAndrés-Galiana, J. Fernández-Martínez, S. González;*
Universidad de Oviedo, Oviedo, Spain.

Introduction: Idiopathic inflammatory myopathies comprise a heterogeneous group of disorders characterized by muscle inflammation that include Polymyositis (PM) and Inclusion Body Myositis (IBM). PM is considered as a T cell-mediated autoimmune disease and IBM is characterized by a combination of T cell-mediated disease and myofiber degeneration.
Materials and Methods: The most discriminatory genes of IBM and PM phenotypes were determined using previously reported microarray data from muscle biopsies of patients by using a combination of fold change, Fisher's Ratio and a new machine learning algorithm.
Results: Our data support the well-known relevance of Major Histocompatibility Complex (MHC) class I molecules in PM and IBM, accessory molecules involved in MHC class I presentation and function, as well as transcription factors that promote cytotoxic CD8 T cell-mediated immune responses, such as STAT1, IRF7 and IRF9,. Additionally, our study unveils the potential relevance of novel genes involved in the pathogenesis of PM, including *OSBPL10*, *GOLM1* and *SIK1*; and IBM (*RPS4Y*, *S100A4*, *S100A6* and *CAPN3*).
Conclusions: IBM and PM remain as incurable autoimmune diseases. Our study confirms the relevance of molecules and pathways widely accepted to play a role in inflammatory myopathies, but it also provides new insights into the pathogenesis of PM and IBM. The role in inflammatory myopathies of the new molecules detected in our study deserves future investigation since they may be potential new therapeutic targets in these type of diseases.
This work was supported by the Spanish grant of Instituto de Salud Carlos III (PI16/01485) and FEDER European Union.

P.C2.03.15

Prevalence and age association of autoantibodies in a Greek healthy adult cohort

*K. Ampelakiotou, E. Synodinou, K. Soufleros, S. Pomoni, A. Lemoni, **A. Tsirogianni**;*
Immunology-Histocompatibility Dept. "Evaggelismos" General Hospital, Athens, Greece.

The aim of this study was to investigate the prevalence of autoantibodies (aabs) in 187 healthy adults classified in 3 groups: group I (18-40 years), group II (41-64) and group III (65-87). Anti-nuclear (ANA), anti-dsDNA, anti-neutrophil cytoplasmic (ANCA), anti-mitochondrial (AMA), anti-smooth muscle (ASMA), anti-liver/kidney microsomal type 1 (anti-LKM1) and anti-parietal cells (APCA) aabs, were studied. Indirect Immunofluorescence (IIF) assay was used initially while positive samples, by Line Immunoassay (LIA), were further analyzed. Positive ANA were detected in 31 of 187 samples (16,6%), 25 out of 116 women (21,6%) and 6 out of 71 men (8,5%). In group I, 10 out of 67 (14,9%) samples, in group II, 12 out of 73 (16,4%) and in group III, 9 out of 47 (19,1%). Anti-dsDNA were not detected in any sample while in only one ANA positive sample anti-Mi-2 were identified. Regarding ANCA, atypical pattern (a-ANCA) was observed in 5 (2,7%) samples without anti MPO/PR3 specificity. ASMA were detected in 3 samples (1,6%), APCA in 9 (4,8%), whereas AMA and LKM1 were not identified. In this studied cohort the sera of 44 (23,5%) individuals had at least one autoantibody while 3 (1,6%) had more than one. In conclusion, it is suggested that in Greek healthy adults the prevalence of autoantibodies increases with age. However, it is lower than in other studies performed worldwide. This might mean that genetic and epigenetic factors, HLA, diet and climate in particular, affect our findings.
*This study has been submitted to the AESKU IFA Prize Competition 2017.

P.C2.03.16

HLA typing and autoantibodies screening among family members of Celiac Disease Greek patients

*V. Kitsiou, D. Kouniaki, K. Soufleros, T. Athanassiades, K. Tarassi, K. Ampelakiotou, S. Pomoni, E. Synodinou, D. Siampani, **A. Tsirogianni**;*
Immunology-Histocompatibility Dept. "Evaggelismos" General Hospital, Athens, Greece.

Introduction: Celiac Disease (CD) is an autoimmune disease caused by genetically induced intolerance to gluten protein that leads to small bowel enteropathy. It affects 1-2% of Caucasoids however is more common in family members (FMs) of CD patients (pts).
The aim of this study was the assessment of HLA typing and autoantibodies screening in the determination of familiar CD prevalence, in a Greek cohort.
Subjects-Methods: The study included 29 recently diagnosed CD pts and 101 asymptomatic first-degree FMs. HLA-DQA1*/DQB1* typing by high resolution PCR-SSP techniques, anti-tissue transglutaminase (tTG) by ELISA and anti-endomysial (EMA) autoantibodies (Aabs) by IIF, were performed.
Results: At least one CD predisposing HLA allele was typed in 28/29 (96.5%) pts and 77/101 (76.2%) FMs. Among them 86 (66.1%) were DQ2, 12 (9.2%) DQ8 and 7 (5.3%) DQ2/8 double positive. All pts and 21 FMs were positive in at least one of the tested Aabs (100% tTG and 96% EMA) and all of them but one pt carried a high risk HLA allele. The study revealed 21 new CD cases (4 parents, 10 offsprings,7 siblings) according to ESPGHAN diagnostic criteria, all possessing both genetic and serological CD markers.
Conclusions: In our cohort the prevalence of CD among first-degree relatives appears higher (20.8%) than in the general population. The fact that these pts remain undiagnosed may lead to severe complications. Therefore, a screening strategy with HLA genotyping as well as tTG and EMA Aabs serological testing, could be strongly recommended in FMs of CD pts.

P.C2.03.17

ANCA-associated Vasculitis: Therapy is the lesser evil

***G. Van Hulst**, L. C. Van Eyndhoven, J. Potjewijd, J. P. Aendekerk, P. van Paassen, J. Damoiseaux, N. de Wit, J. Vanderlocht;*
Maastricht University Medical Centre, Maastricht, Netherlands.

Introduction: The currently applied one-size-fits-all therapeutic approach causes variable levels of immunosuppression between patients due to individual pharmacodynamics and pharmacokinetics. Althoughimmunosuppressive regimens improved overall survival and mortality rates in ANCA-associated vasculitis (AAV), it requires close monitoring of the patients to manage toxicity and prevent life-threatening complications. A paradigm shift to personalized protocols is needed to prevent complications in the future. Therefore, we aim to establish a readout for the level of immunosuppression and secondary risk of complications.
Methods: We compared immune status differences of AAV patients treated with rituximab or azathioprine, during active disease, and age matched healthy controls. Lymphocyte subsets were quantified using standardized 8-color flow cytometry conform the Euroflow consortium. Proliferative capacity of T cells was estimated by flow cytometric analysis of CFSE dilution after polyclonal stimulation. Cytokine secretion profiles will be measured by flow cytometric bead array performed on the culture supernatants.
Results: Our preliminary data indicate that naïve helper T cells and B cells were reduced in rituximab and azathioprine patients compared to age-related controls. Additionally, cytotoxic T cells were decreased by azathioprine whereas relatively unaffected by rituximab.
Discussion: Although underlying mechanisms of both therapies are dissimilar, the similarity of the effect on naïve helper T cells and B cells may motivate the effectiveness of both therapies in the treatment AAV. Interestingly, the dissimilarity of both therapies on cytotoxic T cells could indicate a lesser role for cytotoxic T cells in disease activity of AAV.

P.C2.04 Immune signaling and therapy in autoimmunity - Part 4

P.C2.04.01
Anti-TNFá therapeutics differentially affect *Leishmania* infection of human macrophages

*K. Arens, C. Filippis, H. Kleinfelder, A. Goetzee, G. Reichmann, P. Crauwels, Z. Waibler, **K. Bagola**, G. van Zandbergen;*
Paul-Ehrlich-Institut, Langen, Germany.

Tumor necrosis factor α (TNFα) drives the pathophysiology of human autoimmune diseases and consequently, neutralizing antibodies (Abs) or Ab-derived molecules directed against TNFα are essential therapeutics. As treatment with several TNFα blockers has been reported to entail a higher risk of infectious diseases such as leishmaniasis, we established an *in vitro* model based on *Leishmania*-infected human macrophages, co-cultured with autologous T-cells, for the analysis and comparison of anti-TNFα therapeutics. We demonstrate that neutralization of soluble TNFα (sTNFα) by the anti-TNFα Abs Humira®, Remicade® and its biosimilar Remsima® negatively affects infection as treatment with these agents significantly reduces *Leishmania*-induced T-cell proliferation and increases the number of infected macrophages. In contrast, we show that blockade of sTNFα by Cimzia® does not affect T-cell proliferation and infection rates. Moreover, compared to Remicade®, treatment with Cimzia® does not impair the expression of cytolytic effector proteins in proliferating T-cells. Our data demonstrate that Cimzia® supports parasite control through its conjugated polyethylene glycol (PEG) moiety as PEGylation of Remicade® improves the clearance of intracellular *Leishmania*. This effect can be linked to complement activation, with levels of complement component C5a being increased upon treatment with Cimzia® or a PEGylated form of Remicade®. Taken together, we provide an *in vitro* model of human leishmaniasis that allows direct comparison of different anti-TNFα agents. Our results enhance the understanding of the efficacy and adverse effects of TNFα blockers and they contribute to evaluate anti-TNFα therapy for patients living in countries with a high prevalence of leishmaniasis.

P.C2.04.02
Role of GSK-3 in the IL-10 production of marginal zone B cells

***B. Barátki**[1], D. Kövesdi[2];*
[1]Department of Immunology, Eötvös Loránd University, Budapest, Hungary, [2]MTA TKI, Budapest, Hungary.

Glycogen synthase kinase 3 (GSK-3) is a constitutively active serine/threonine kinase. In macrophages signalling through the IFN-γ receptor antagonizes Akt/PKB activation thus blocks the TLR-induced IL-10 production on a GSK-3-dependent manner. It is unclear however, how GSK-3 is involved in the IL-10 expression of B lymphocytes. We showed previously that marginal zone (MZ) B cells, unlike follicular (FO) B cells, produced a high amount of IL-10 under inflammatory conditions. Therefore, in our recent work we aimed to investigate the involvement of GSK-3 in the BCR, TLR9 and IFN-γ receptors induced IL-10 production of MZ B cells.Throughout our experiments we used MZ and FO B cells isolated from the spleens of DBA/1 mice and stimulated simultaneously with anti-IgM, CpG and IFN-γ in the presence or the absence of a GSK-3 inhibitor. The effects of GSK-3 and its inhibitor on IL-10 production were analysed by Western blot, flow cytometry, real-time RT-PCR and IL-10 specific ELISA.
Our results showed that simultaneous signals through the BCR, TLR9 and IFN-γ receptors had a synergistic effect on IL-10 expression. Using the GSK-3 inhibitor we proved the impact of GSK-3 on the IL-10 production of MZ B cells and demonstrated its prolonged versus transient phosphorylation/inactivation in MZ and FO B cells, respectively.
We assume that the transient inactivation of GSK-3 is needed for the proper induction of IL-10 production and for the regulatory differentiation of MZ B cells under inflammatory condition.
This project was supported by the MTA Premium Post Doctorate Research Program.

P.C2.04.03
The mechanism of RNA-binding protein HuR regulating Th17 cell differentiation

***J. Chen**, S. Yu;*
Thomas Jefferson University, Philadephia, United States.

T helper 17 (Th17) cells play critical pathogenic roles in several autoimmune and inflammatory diseases including multiple sclerosis and experimental autoimmune encephalomyelitis (EAE). It is well-known that IL-6 in the presence of TGF-b promotes Th17 cell differentiation. Although cytokine-mediated transcriptional regulation of Th17 cell differentiation and proliferation is fully investigated, it remains unknown about post-transcriptional regulation of Th17 cell differentiation and proliferation by RNA-binding proteins (RBPs). We previously demonstrated that the RBP HuR (ELAVL1) post-transcriptionally modulates expressions of IL-17, GM-CSF and CCR6 in Th17 cells. Here we provided new data that demonstrated that HuR upregulates phosphorylation of Stat3 and RoRrt expression to promote Th17 cell differentiation and proliferation using the HuR conditional knockout (KO) mice. Mechanically, HuR protein stabilizes IL-6R mRNAs leading to increased its mRNA half-life and protein level, therefore, enhanced IL-6/IL-6R signaling and phosphorylation of Jak1 and Stat3, ultimately, increased RoRrt expression. Taken together, these results suggested that HuR positively orchestrates Th17 cell differentiation and proliferation. Accordingly, HuR might be a novel therapeutic target for treatment of Th17 cell-mediated autoimmune diseases. (Supported by NIH R01 AI119135)

P.C2.04.04
Increased alternative pathway regulation by using a complement regulator factor H potentiating antibody

***G. Dekkers**[1], R. B. Pouw[1,2], M. C. Brouwer[1], M. De Gast[1], A. E. Van Beek[1,2], P. Sánchez-Corral[3,4], L. Van Den Heuvel[5,6], C. Q. Schmidt[7], A. Van Der Ende[8], D. Wouters[1], T. W. Kuijpers[2,9], T. Rispens[1], I. Jongerius[1];*
[1]Sanquin Research, Department of Immunopathology, Amsterdam, The Netherlands; and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands, [2]Department of Pediatric Hematology, Immunology & Infectious Diseases, Emma children's hospital, Academic Medical Center Amsterdam, Amsterdam, Netherlands, [3]Centre for Biomedical Network Research on Rare Diseases (CIBERER), Madrid, Spain, [4]Complement Research Group, Research Unit, University Hospital, Madrid, Spain, [5]Laboratory of Pediatric Nephrology, Department of Development & Regeneration, KU Leuven, Leuven, Belgium, [6]Department of Pediatric Nephrology, Radboud University Medical Center, Nijmwegen, Netherlands, [7]Institute of Pharmacology of Natural Products and Clinical Pharmacology, Ulm University, Ulm, Germany, [8]Department of Medical Microbiology, the Netherlands Reference Laboratory for Bacterial Meningitis, CINIMA, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands, [9]Sanquin Research, Department of Blood Cell Research, Amsterdam, The Netherlands; and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands.

Heterozygous mutations in the soluble complement regulator factor H (FH) are commonly associated with severe complement-related diseases such as atypical hemolytic uremic syndrome (aHUS) and age-related macular degeneration. We have discovered an anti-FH monoclonal antibody (mAb) that enhances FH function which could be important for clinical use. In ELISA, by measuring C3 deposition on LPS-coated surfaces, the potentiating anti-FH mAb could inhibit alternative pathway-mediated complement activation. Importantly, the mAb neither affects FH cofactor activity in fluid phase, nor causes inhibition by consumption of circulating C3. Addition of the potentiating mAb prevents complement-mediated hemolysis of sheep erythrocytes induced by aHUS patient sera. Moreover, C3b deposition on human endothelial cells (HUVEC) incubated with patient sera is prevented by enhancing FH function with the mAb. In both ELISA and surface plasmon resonance (SPR), binding of the mAb to FH increased the affinity of FH for C3b. And the degeneration of the alternative pathway convertase (C3bBb) by FH is increased. Studying recombinant FH with aHUS mutations shows that the mAb also potentiates FH function of these mutated FH. In contrast to Eculizumab, a well-known complement inhibitor used in the clinic, our mAb did not affect the bactericidal activity of normal human serum against *Escherichia coli* or *Neisseria meningitidis* and might therefore be a safer alternative to treat complement mediated diseases. In conclusion, our unique potentiating anti-FH mAb might serve as an alternative therapeutic for complement-mediated diseases to inhibit unwarranted complement activation on human endothelial cells while maintaining complement-mediated clearance of bacteria.

P.C2.04.05
Understanding the importance of B lymphocytes in the development of Type 1 Diabetes

***L. Egia-Mendikute**[1], B. Arpa[1], E. Rosell-Mases[1], M. Corral-Pujol[1], C. Vived[1], A. Panosa[1], C. Mora[1], J. Carrascal[1], T. Stratmann[2], D. Serreze[3];*
[1]University of Lleida & IRBLleida, Lleida, Spain, [2]University of Barcelona, Barcelona, Spain, [3]The Jackson Laboratory, Bar Habor, Maine, United States.

Introduction: To analyze through which mechanisms B lymphocytes participate in Type 1 Diabetes (T1D) development, we have generated two B lymphocyte transgenic mice, the 116C-NOD and the NOD-PerIg. In 116C-NOD mice, which wears immunoglobulins reactive to a non yet defined ß-cell autoantigen, late disease onset and a decrease of disease incidence is found in both genders compared to the wild-type NOD mice. In contrast, the NOD-PerIg mouse, in which the Immunoglobulins have specificity for peripherin, an acceleration of T1D onset and an increase of disease incidence are observed in both genders.
Materials Methods: Phenotypic flow cytometry analysis, proliferation and cytokine release assays were performed on B and T lymphocytes from mechanically disrupted spleens and pancreatic islets of prediabetic NOD, 116C-NOD and NOD-PerIg mice.
Results: Significant functional and phenotypic differences were observed between both transgenic mouse models. Islet-infiltrating B-lymphocytes (IIBLs) from 116C-NOD displayed lower expression of FAS, CD86, H-$2K^d$ and H-$2IA^{g7}$ compared to NOD mouse, whereas increased expression of the same molecules was detected in IIBLs from NOD-*PerIg*. Moreover, the number of IIBLs expressing BAFF, BAFF-R, and TACI molecular markers was also higher in 116C-NOD compared to NOD and NOD-*PerIg* mice. Moreover, proliferation assays have showed high capacity of NOD-*PerIg* mice B cells to produce large amounts of cytokines and to induce T cell activation, compared to the other models.
Conclusions: In NOD-*PerIg* mice, B-lymphocytes have an activated phenotype and support accelerated T1D development. In 116C-NOD mice, B lymphocytes display an anergic like phenotype, delaying T1D onset and decreasing disease incidence.

P.C2.04.06

Signaling induced by PRL in immature B cells from systemic lupus erythematosus mice

R. Flores[1], *F. Blanco*[1], *E. Fuentes*[2], *A. Pizaña*[3], *P. Gorocica*[3], *L. Chavez*[1], *K. Chavez*[1];
[1]*IMSS, Mexico, Mexico,* [2]*Hospital Infantil de Mexico, Mexico, Mexico,* [3]*Instituto Nacional de Nutricion, Mexico, Mexico.*

Introduction. The ontogeny of the B lymphocyte begins in bone marrow, where the precursor cells pass through different stages of maturation until they become immature B lymphocyte. In this stage, the auto-reactive clones are eliminated by mechanisms of central tolerance, such as the editing of the receptor or death by apoptosis. The PRL receptor has been shown to be expressed on immature B lymphocytes from systemic lupus erythematosus mice and WEHI-231 cells (the WEHI-231 cell line, it has been widely used as a model for the study of tolerance). When these cells incubated with this hormone, a decrease in the apoptosis was found. Prolactin (PRL) is a globular protein composed of 199 amino acids. The PRL receptor belongs to the type I cytokine receptor family. The binding of PRL with its receptor activates the JAK-STAT, MAPK and PI3K-AKT signaling pathways. Objective. Determine the signaling pathways induces by PRL in immature B cells from systemic lupus erythematosus mice. Methodology. Immature B cells from mice and WEHI-231 cells were incubated with PRL for 30 minutes. Then the cells were labelled with anti-STAT-1, STAT-3, STAT-5, AKT, and ERK1/2 phosphorylated antibodies, and IMF was determined by flow cytometry. Results. Immature B cells from systemic lupus erythematosus incubated with PRL increased the activation only of STAT-3, and WEHI-231 cells increased the activation of STAT-3 and AKT. Conclusions. The binding of PRL to its receptor activates only STAT-3 in immature B cells from lupus mice, and activates STAT-3 and AKT in WEHI-231 cells.

P.C2.04.07

Interest of anti-aquaporine 4 in the etiological diagnosis of central nervous system inflammatory diseases

S. Mejdoub[1], *S. Feki*[1], *M. Dammak*[2], *N. Farhat*[2], ***H. Hachicha***[1], *C. Mhiri*[2], *H. Masmoudi*[1];
[1]*Immunology Laboratory, Habib Bourguiba Hospital, Sfax, Tunisia,* [2]*Neurology Department, Habib Bourguiba Hospital, Sfax, Tunisia.*

Neuromyelitis optica (NMO) is an inflammatory demyelinating disease of the central nervous system (CNS) with a predilection for optic nerves and spinal cord. Anti-aquaporine 4 antibodies (anti-AQP4) represent a specific biomarker of NMO and its borderline forms known as NMO spectrum disorders (NMOSD). The aim of our study was to evaluate the seroprevalence and the clinical interest of this marker in case of CNS inflammatory disorder in Tunisian patients.
In our study, anti-AQP4 was screened in the serum of 126 patients with CNS inflammatory disorder using indirect immunofluorescence on transfected cells (Euroimmun®, Germany).
Our results showed that anti-AQP4 seroprevalence was 3,9%. The 5 seropositive patients were women and had a mean age of 40 years (26-56). Three patients had an optico-spinal impairment and two had an isolated myelitis. It was the first clinical episod for 3 patients/5. In one case of optico-spinal impairment, seropositivity was detected in a second sample performed one year after an initial seronegativity. The diagnosis of NMOSD was made for the 5 cases.
In conclusion, it seems that anti-AQP4 has a low seroprevalence in our country. However, this marker appears to be clinically relevant for NMOSD diagnosis, especially in case of incomplete and atypical presentation. We also demonstrate the interest of repeating anti-AQP4 screening after an initial seronegativity in front of highly suggestive clinico-radiological features.

P.C2.04.08

NIK-IKK complex controls NF-êB-dependent inflammatory activation of the endothelium in response to LTâR ligation

P. Kucharzewska[1], *C. X. Maracle*[2], ***K. C. M. Jeucken***[2], *J. van Hamburg*[2], *S. W. Tas*[2], *H. Olsson*[1];
[1]*AstraZeneca R&D, Gothenburg, Sweden,* [2]*AMC Amsterdam, Amsterdam, Netherlands.*

Background: Endothelial cells (EC) are important contributors to inflammation via expression of inflammatory mediators, including chemokines and adhesion molecules. Production of inflammatory mediators can be induced via lymphotoxin-β receptor (LTβR)-ligation, resulting in activation of canonical and NF-κB-Inducing Kinase (NIK)-dependent noncanonical NF-κB signaling. However, the relative contribution of the individual NF-κB pathways to inflammatory activation of EC is largely elusive.
Objective: To identify the molecular pathways by which LTβR-ligation drives inflammatory activation of EC.
Methods: EC were stimulated with LTβ or LIGHT to activate LTβR, followed by analysis of downstream NF-κB signaling pathways and expression of inflammatory mediators. To repress canonical NF-κB signaling an IKKβ-inhibitor was used, and noncanonical NF-κB signaling was repressed using siRNAs targeting NFκB2. The role of NIK in LTβR signaling was investigated using inhibitors and siRNAs targeting NIK and overexpression of NIK.
Results: LTβR-ligation resulted in activation of canonical and noncanonical NF-κB signaling, and subsequent expression of inflammatory mediators. IKKβ inhibition repressed LTβR-induced inflammatory activation of EC, indicating that this process was mediated through canonical NF-κB signaling. Interestingly, NIK targeting also decreased LTβR-induced expression of inflammatory mediators, while targeting NFκB2 had no effect. Further analyses, including silencing and overexpression of NIK, demonstrated a clear role for NIK in activation of canonical NF-κB signaling by amplifying IKK complex activity.
Conclusions: These findings suggest that NIK can serve as an amplifier of canonical NF-κB signaling and associated inflammatory responses in EC, which may play an important role in the inflammatory process. Consequently, NIK may be an attractive therapeutic target.

P.C2.04.09

Humanized anti-FH monoclonal antibodies as a new and robust standard and control for anti-FH ELISA

K. A. Gelderman[1], ***A. M. Kamp***[2], *M. C. Brouwer*[2], *L. A. Trouw*[3], *R. B. Pouw*[2], *D. Wouters*[2];
[1]*Sanquin Diagnostic Services, Amsterdam, The Netherlands, Amsterdam, Netherlands,* [2]*Sanquin Research, Dept of Immunopathology, Amsterdam, The Netherlands and landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands, Amsterdam, Netherlands,* [3]*Dept of Rheumato;ogy, Leiden Univercity Medical Center, Leiden, The Netherlands, Amsterdam, Netherlands.*

Genetic variants of regulator alternative pathway regulator Factor H (FH) are strongly associated with renal diseases like atypical HUS (aHUS) and C3-glomerulonephritis (C3GN). Next to these genetic variants, anti-factor H (FH) auto-antibodies are detected in 10% to 50% of these patients. In aHUS these auto-antibodies are associated with extra-renal features and their presence mostly coincides with a deletion of the *CFHR1/3* genes. In C3G, in contrast, the anti-FH antibodies target different epitopes and the presence of anti-FH is not associated with a deletion of the *CFHR1/3* genes. To detect anti-FH an ELISA is widely performed using full-length FH as antigen. Anti-FH detection is performed either for diagnostic purposes or for follow-up. To compare samples and to quality control individual tests, each test requires a reliable and reproducible standard line and controls. Positive patient material is commonly used, but availability is limited and patient-to-patient variation significant. To solve this, we humanized two FH mouse monoclonal antibodies, to produce stable and comparable human anti-FH antibodies that can serve as standard and/or assay control. Two monoclonals were chosen that recognize domain 20 of FH, being representative for patient auto-antibodies that often are directed against this domain. These humanized antibodies recognized FH and the dilution curves were parallel to those of antibody-positive patient samples and to a internationally used plasmaferesis material standard. In conclusion; we made two humanized anti-FH clones that produce antibodies to use as standard and control in an anti-FH ELISA. This allows international standardization and comparability of the anti-FH test.

P.C2.04.10

Neutrophils display features of necroptosis in granulomatosis with polyangiitis

T. Stallbaum, ***A. Kerstein****, G. Riemekasten, A. Müller, P. Lamprecht;*
Department of Rheumatology and Clinical Immunology, Luebeck, Germany.

Introduction Dysregulation of cell death plays a crucial role in the initiation of chronic inflammation and autoimmunity. Cardinal pathological features of granulomatosis with polyangiitis (GPA) are necrotizing granulomatous inflammation and necrotizing autoimmune vasculitis. Here we provide evidence, that necroptosis, *i.e.* a regulated form of inflammation-inducing necrosis, is involved in the pathophysiology of GPA.
Methods Polymorphonuclear leukocytes (PMN) were isolated from peripheral blood of GPA patients and healthy controls (HC) (n = 10, each). mRNA and proteins were extracted. Gene and protein expression of markers of apoptosis (caspase 8) and necroptosis [receptor interacting serine/threonine kinase 1 (RIP1); RIP3; cellular inhibitor of apoptosis protein-1/2 (cIAP1/2); X-linked inhibitor of apoptosis protein (XIAP); mixed lineage kinase domain like pseudokinase (MLKL)] were determined by qPCR and western blot.
Results All markers of cell death, including necroptosis, were expressed by PMN of both GPA and HC in terms of mRNA and protein. There were no significant differences between GPA and HC regarding gene expression. However, there was a decreased protein expression of cleaved caspase 8 as well as an increased expression of apoptosis inhibitors cIAP2 and XIAP, demonstrating blocked apoptosis in GPA PMN. In contrast, necroptosis executers RIP3 and MLKL showed an increased expression in GPA PMN.
Conclusion Our results reveal necroptosis as a relevant form of cell death by PMN in GPA. Given the impact of dying neutrophils for the induction of chronic inflammation and autoimmunity, regulated necroptosis could represent a potential therapeutic target in GPA.

P.C2.04.11

CXCL4 in Tunisian patients with systemic sclerosis

L. Laadhar[1], *I. Nammouchi*[1], *I. Ben Ghorbel*[2], *I. Ayadi*[1], *H. Lahmar*[1], *M. H. Houman*[2], *M. Kallel-Sellami*[1];
[1]*Immunology Department, la Rabta hospital, Tunis, Tunisia,* [2]*Internal Medicine department, la Rabta hospital, Tunis, Tunisia.*

Introduction: We aimed, first to assess the CXCL4 level in Tunisian patients with systemic sclerosis (SSc) comparing to healthy controls and to patients presenting other clinical conditions; and second to search correlations between the level of CXCL4 and the clinical manifestations of the disease. Methods: We enrolled 50 patients with SSc and with no other connective tissue diseases associated. All patients met the 2013 ACR/EULAR classification criteria. We also recruited 30 age- and sex-matched healthy controls, 36 patients with systemic lupus erythematosus (SLE), 30 patients with rheumatoid arthritis (RA) and 27 patients with Sjögren's syndrome (SS). Levels of CXCL4 were determined using ELISA (R&D Systems®). Results: Patients were 47 women and 3 men with a mean age of 50.1 years. The mean level of CXCL4 in patients with SSc was 47.80 ± 18.27ng / ml. It was significantly higher than that in healthy controls (38.34 ± 15.83ng / ml), in patients with SLE (25.46 ± 16.09 ng / ml) and patients with RA (39.28 ± 11.91ng / ml) ($p = 0.021$, <0.001 and $=0.026$ respectively). There was no significant difference between the mean level of CXCL4 in patients with SSc and in patients with SS. There was no statistically significant correlation between CXCL4 levels and the various clinical manifestations of the disease. Conclusion: CXCL4 appears as a potential biomarker for SSc, however it is not correlated to the clinical phenotype of the disease in our cohort.

P.C2.04.12

Bruton's Tyrosine Kinase (BTK) regulates the NLRP3 inflammasome directly through NLRP3 tyrosine phosphorylation

X. Liu*;*
Department of Immunology, Tübingen, Germany.

The NLRP3 inflammasome is an inflammatory machinery participating in the pathogenesis of many inflammatory diseases. Its activation involves NLRP3 recruiting the adaptor ASC, caspase-1 binding and auto-proteolytic activation and finally IL-1β and IL-18 maturation. However, the regulatory mechanisms of this vital inflammatory process are poorly understood. In previous studies, we identified BTK as a novel regulator of the NLRP3 inflammasome. Pharmacological and genetic BTK ablation attenuated caspase-1 activation and IL-1β maturation. BTK directly interacted with NLRP3 and ASC.
In the current study, we further found that tyrosine phosphorylation of NLRP3 was significantly enhanced by the presence of BTK in co-transfected HEK293T cells, and this phosphorylation was diminished by BTK inhibitors, but not the NLRP3 inhibitor MCC950.
Furthermore, a time-course pattern of increased tyrosine phosphorylation of endogenous NLRP3 was observed in human and mouse primary cells in response of Nigericin. However, NLRP3 phosphorylation was reduced in immune cells from genetically deficient human XLA patients and *Btk* knockout mice. Using truncated and tyrosine-mutated NLRP3 constructs, we hope to identify the exact phosphorylation site of NLRP3 that BTK targets and work out how NLRP3 tyrosine phosphorylation by BTK regulates inflammasome activity. Overall, our data contribute to a better understanding of the BTK regulation mechanism of NLRP3 activation. Given the clinical availability of FDA-approved BTK inhibitors this may pave the way for new treatment strategies in NLRP3 inflammasome-linked inflammation.

P.C2.04.13

Down-modulation of autoreactive B-cells by protein-engineered chimeric molecules in mouse model of Type 1 Diabetes

I. Manoylov[1], *G. Boneva*[1], *N. Mihaylova*[1], *I. Doytchinova*[2], *A. Tchorbanov*[1,3];
[1]*The Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria,* [2]*Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria,* [3]*National Institute of Immunology, Sofia, Bulgaria.*

Introduction: Type 1 diabetes (T1D) is characterized by autoimmune attack against the insulin-producing beta-cells of the pancreas. One of the main beta-cells' autoantigens in T1D is glutamic acid decarboxylase (GAD65) - a membrane-bound enzyme catalyzing the formation of gamma-aminobutyric acid. Autoreactive B lymphocytes play major role in the pathogenesis of the disease. They produce autoantibodies against several autoantigens. B cells can activate T cells and can modulate the immune response via cytokine production. Thus, eliminating autoreactive B lymphocytes may serve as a potential treatment against T1D. Downregulation of murine B cells is accomplished via the activation of the negative receptor Fc-gammaRIIB. Logically, this receptor could be a potential target for suppression of autoreactive B lymphocytes.
Materials and methods: We constructed chimeric protein molecules, containing a monoclonal antibody specific for the mouse inhibitory receptor Fc-gammaRIIB, coupled to peptide epitopes derived from GAD65 protein. The ability of these molecules to modulate the immune response was tested in an induced murine model of T1D. The parameters of this interaction were characterized by FACS analysis, ELISpot and proliferation assays.
Results: The chimeric molecules, presented in this study, bind GAD65 - specific B-lymphocytes and suppress selectively their proliferation by co-crosslinking of the inhibitory Fc-gammaRIIB and the BCR.
Conclusions: The aim of our study was to construct chimeric molecules, using antibody against Fc-gammaRIIB conjugated to GAD65 epitopes. The chimeric molecules were expected to suppress specifically autoreactive B-cells in a mouse model of T1D. This treatment presents a novel specific therapy for autoimmune diabetes.

P.C2.04.14

The macrophage migration inhibitory factor pathway in human B cells: tight control and dysregulation in multiple sclerosis

L. Rijvers[1], *M. Melief*[1], *R. M. van der Vuurst de Vries*[2], *M. Stéphant*[1], *J. van Langelaar*[1], *A. F. Wierenga-Wolf*[1], *J. M. Hogervorst*[1], *A. Geurts-Moespot*[3], *F. C. Sweep*[3], *R. Q. Hintzen*[1,2], *M. M. van Luijn*[1];
[1]*Department of Immunology, MS Center ErasMS, Erasmus MC, Rotterdam, Netherlands,* [2]*Department of Neurology, MS Center ErasMS, Erasmus MC, Rotterdam, Netherlands,* [3]*Department of Chemical Endocrinology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands.*

In multiple sclerosis (MS), B cells survive peripheral tolerance checkpoints to mediate local inflammation, but the underlying molecular mechanisms are underexplored. In mice, the macrophage migration inhibitory factor (MIF) pathway controls B-cell development and the induction of experimental autoimmune encephalomyelitis. How the MIF pathway is controlled in human B cells and contributes to disease onset in MS patients remains highly elusive.
Here, we found that MIF receptor CD74 was downregulated, whereas MIF receptor CXCR4 was upregulated in B cells of clinically isolated syndrome (CIS) patients who rapidly develop MS (n=16) as well as clinically definite MS patients (n=15). Transitional and naive mature B cells displayed the highest CXCR4/CD74 expression ratios compared to class-switched and non–class-switched memory subsets in these patients, implying that this $CXCR4^{high}CD74^{low}$ phenotype reflects a more immature state of B cells. Interestingly, B cells were identified as the main immune subset in blood expressing MIF, which was downregulated in early MS patients. Blockade of MIF/CD74 signaling triggered CXCR4 expression, while blockade of MIF/CXCR4 signaling upregulated CD74 on activated B cells. Moreover, CD74-blocked B cells were less able to proliferate and to express pro-inflammatory genes *NFKB1, IL6* and *TNF*. On the contrary, CXCR4-blocked B cells showed increased sensitivity to Fas-mediated apoptosis.
This study points to the existence of a functionally relevant regulation loop between MIF, CD74 and CXCR4 in human B cells. The disturbance of this loop during MS onset provides new insights into how pathogenic B cells survive peripheral tolerance checkpoints in autoimmune diseases such as MS.

P.C2.04.15

Evaluation of MAPK3 in the context of CD25 expression on natural Tregs for the immune tolerance development in patients with recurrent pregnancy loss

R. Susurkova[1], *A. Velichkov*[1], *M. Muhtarova*[2], *M. Guenova*[2], *I. Antonova*[3], *G. Nikolov*[3], *A. Mihova*[4], *V. Terzieva*[1];
[1]*Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratanov", Sofiya, Bulgaria,* [2]*National Specialised Hospital for Active Treatment of Haematological Diseases, Sofiya, Bulgaria,* [3]*Center for Reproductive Biology and Medicine "Reprobiomed", Sofiya, Bulgaria,* [4]*University Hospital Lozenetz, Sofiya, Bulgaria.*

Introduction: The establishment of an immune tolerant milieu is a *conditio sine qua non* for the development of pregnancy. Among multiple factors involved, the population of regulatory T cells (Tregs) is of critical importance. A particular subset of Tregs, natural Tregs (nTregs), is shown to be impaired in women with recurrent pregnancy loss (RPL). The present study is aimed at evaluating the impact of MAPK3 signaling molecule in this process. Materials and Methods: PBMCs from 10 patients with RPL (24-37yrs) and 10 age-matched controls (HC) with history of successful pregnancy were stained with anti-CD3/CD4/CD45RA/CD25/FoxP3/MAPK3 antibodies either non-stimulated or stimulated via IL-2 or anti-CD3/CD28. MAPK3 was assessed by flowcytometry/PrimeFlow™ and confocal microscopy. FACS and statistical analyses were done by FlowJoV10 and GraphPad7 software. Results: The proportion of nTregs in patients was found decreased in comparison to HC ($p<0.05$). In controls, but not in patients, the majority of cells were CD25+ ($p<0.05$). The evaluation of MAPK3 in study groups showed differences between CD25+ and CD25- subsets expressed mainly in anti-CD3/CD28 stimulated cells that were further evidenced by the confocal microscopy. Conclusions: Our results indicate variations in MAPK3 expression that might be associated with the expression of CD25 on nTregs. Further experiments are envisaged to precisely analyze their impact on nTregs for the immune tolerance establishment. Acknoligments:"Program for Support of Young Researchers and PhD Students at the Bulgarian Academy of Sciences (Grant no. 17-118/2017)".

P.C2.04.16

Balancing JAK/STAT-signalling with tofacitinib in monocytes of healthy controls and IBD patients

*F. Cordes[1], T. Weinhage[2], E. Lenker[2], D. Bettenworth[1], C. Kessel[2], D. Föll[2], **G. Varga**[2];*
[1]Department of Medicine B, University Hospital Muenster, Germany, [2]Department of Pediatric Rheumatology and Immunology, University Hospital Muenster, Germany.

Background. JAK/STAT-blockade by tofacitinib, an oral JAK1/JAK3 inhibitor, promises new treatment options in inflammatory bowel disease (IBD). Monocytes are key players in inflammatory responses and bridge natural and acquired immunity. However, the impact of tofacitinib on monocytes of IBD patients has not been investigated so far. **Aims**: In this study, inhibition of the JAK/STAT-pathway by tofacitinib was investigated in monocytes of IBD patients and healthy controls. **Methods** Primary monocytes from healthy human controls and IBD patients with active disease were analyzed for cytokine expression and phenotype after stimulation with GM-CSF/IFNg and tofacitinib pre-treatment with non-toxic dosages of 1-1000 nM. Furthermore, dose-dependent inhibition of JAK/STAT-phosphorylation in monocytes and their capacity to induce Foxp3$^+$-regulatory T-cells in co-cultures with autologous naïve T-cells was analyzed. **Results** In control monocytes, tofacitinib at concentrations of 10-100 nM did not inhibit JAK2/STAT5-signalling but blocked JAK1/STAT1-pathway. In a pro-inflammatory setting induced by GM-CSF/IFNγ co-stimulation, tofacitinib had the most beneficial effect on control monocytes at 100 nM leading to blockade of pro-inflammatory cytokines TNFα and IL-6, while simultaneously increasing Tregs in co-cultures. In IBD monocytes with GM-CSF/IFNγ co-stimulation, tofacitinib 1000 nM was most anti-inflammatory including inhibition of TNFα and IL-6 with simultaneous restoration of IL-10. Regulation by tofacitinib was significantly stronger in UC than in CD monocytes. **Conclusion**. Tofacitinib dose-dependently facilitates reprogramming of monocytes to a more regulatory phenotype in control and IBD monocytes. The beneficial effect seemingly is selective JAK/STAT blockade with inhibition of pro-inflammatory responses while regulatory pathways stay intact by adequate tofacitinib dosage. <!--EndFragment-->

P.C2.04.17

Selective depletion of MOG-reactive B lymphocytes by modular T cell targeting

***C. S. Weiss**[1], K. Akgün[1], S. Albert[2], M. P. Bachmann[3], T. Ziemssen[4];*
[1]Center of Clinical Neuroscience, Department of Neurology, University Hospital, 'Carl Gustav Carus' Technische Universität Dresden, Dresden, Germany, [2]University Cancer Center, Dresden, Tumor Immunology, Carl Gustav Carus Universitätsklinikum Dresden, Dresden, Germany, [3]Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Tumor Immunology, UniversityCancerCenter (UCC), 'Carl Gustav Carus' Technische Universit€at Dresden, Cellex Patient Treatment GmbH, Dresden, Germany, [4]MS Center Dresden, Center of Clinical Neuroscience, Department of Neurology, University Hospital Carl Gustav Carus, Dresden University of Technology, Dresden, Germany.

Multiple sclerosis (MS) is defined as primarily T-cell-mediated disease and initial treatment strategies focused on pathogenic T-cell modulation. Nevertheless, auto-reactive B-cells play a relevant role in MS pathogenesis reflected by myelin-specific auto-antibody production, antigen presentation and pro-inflammatory cytokine release. Novel MS treatment regimens target B-cells using general (CD20) but not antigen-specific antibody-mediated depletion. For depletion of auto-reactive pathogenic B-cells we used a system originally established for tumor-cell-targeting. To circumvent severe side effects that occurred in earlier trials, a novel platform was developed where T-cells are equipped with a universal chimeric antigen receptor (UniCAR).
Instead of binding directly to a certain structure on the target cell surface, UniCAR T-cells can be switched on and off by administering a targeting module (TM) consisting of a peptide epitope recognized by the UniCAR T-cell and a binding moiety directed against a distinct structure on the target cell surface. We determined the extracellular domain of the myelin oligodendrocyte glycoprotein (MOG) antigen as TM for specific depletion of B-cells carrying anti-MOG antibodies. We transduced a human B-cell line with an artificial antibody (scFv MOG) to achieve a constant availability of anti-MOG antibody for *in vitro* studies. We could demonstrate efficient redirection of UniCAR T-cells against scFv MOG-positive B-cells in a strictly target-dependent and target-specific manner. Binding capability of the TM was assessed by flow cytometry. We demonstrate that the UniCAR system is not restricted to cancer therapies and that antigen-specific depletion of auto-reactive B-cells is possible and considered to be used in autoimmune diseases, too.

P.C2.04.18

The culture dish surface influences the phenotype and cytokine production of immunogenic and tolerogenic monocyte-derived dendritic cells

*A. Sauter[1], **D. H. Yi**[2], S. Roersma[2], Y. Li[2], S. Appel[2];*
[1]Department of Biomedicine, Bergen, Norway, [2]Broegelmann Research Laboratory, Bergen, Norway.

Monocyte-derived dendritic cells (moDC) are an important scientific and clinical source of functional dendritic cells (DC). However, the optimization of the generation process has to date mainly been limited to the variation of soluble factors. In this study, we investigated the impact of the cell culture surface on the DC phenotype and cytokine profile. We compared a standard cell culture dish to a non-adherent culture dish for two immunogenic maturation conditions, two tolerogenic conditions and an unstimulated control. Phenotype and cytokine profile were determined after a 3-day culture. Light microscopy revealed an increase in homotypic cluster formation correlated with the use of non-adherent surfaces which could be reduced by using blocking antibodies against CD18. All surface markers analyzed showed moderate to strong differences depending on the surface. Significant differences in the secretion of many cytokines were observed, especially for cells stimulated with LPS. These results provide evidence that the DC phenotype crucially depends on the surface used during moDC generation. This might have an important application in the optimization of DC-based immunotherapy development and underlines that the local surrounding can interfere with the final DC population beyond the soluble factors.
Funding: This research was supported by the European Union 7th Framework Programme as part of the project NanoII, grant agreement number 229289, the Meltzer foundation, the Broegelmann foundation and the Western Norway Regional Health Authorities (grant nr. 912065).

P.C2.04.19

Cytokine productionin peripheral blood cells of patients with Diabetes mellitus type 1

***S. Zivancevic-Simonovic**[1], O. Mihaljevic[1], S. Markovic[1,2], S. Popovic[1], I. Majstorovic[3], O. Milosevic-Djordjevic[1];*
[1]Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia, [2]Clinical Center Kragujevac, Kragujevac, Serbia, [3]Military Medical Academy, Institute for Medical Research, Belgrade, Serbia.

Introduction. Type 1 diabetes mellitus (T1DM) is a T cell-mediated autoimmune disease in which the immune system attacks the insulin producing β cells. The role of cytokines in the pathogenesis of T1DM is still insufficiently clarified. The aim of this study was to analyze cytokine profile in patients with T1DM and to compare it with those in healthy subjects. Materials and Methods**.** The study group included 12 patients with DM type 1 and 20 healthy controls. Cytokine levels were determined in supernatants obtained from unstimulated and phytohemagglutinin (PHA)-stimulated whole blood cultures *in vitro*. The concentrations of selected cytokines: interferon gamma (IFN-γ), interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 10 (IL-10), interleukin 12 (IL-12p70), interleukin 13 (IL-13), interleukin 17A (IL-17A) and tumor necrosis factor alpha (TNF-α) were measured using multiplex cytokine detection systems for Human Th1/Th2/Thl7/Th22. Results. Unstimulated blood cell of T1DM patients produced more IL-6 (1056±752 vs 108±49 pg/mL, p=0.045), IL-10 (168±159 vs 0 pg/mL, p=0.014), IL-17A (142±134 vs 0 pg/mL, p=0.029) and TNF-α (105±41 pg/mL vs 33±37 pg/mL, p=0.029) than controls. The mean production of IL-10 and TNF-α in PHA-stimulated blood cells of T1DM patients tended to be higher than in controls, but statistically significant difference was obtained only for IL-6 (4295±2744 vs 1367±1313 pg/mL, p=0.040). Conclusions: The unstimulated peripheral blood cells of T1DM patients produce more pro-inflammatory cytokines IL-6, IL-17A and TNF-α, and anti-inflammatory cytokine IL-10 than controls. *In yitro* stimulated PBC of T1DM patients produce more proinflammatory cytokine IL-6 than controls.

P.C2.05 Immune signaling and therapy in autoimmunity - Part 5

P.C2.05.01

Influence of autologous apoptotic cell cultures on the parameters of early apoptosis of T-lymphocytes from healthy individuals and patients with rheumatoid arthritis

*T. Y. Abramova[1], V. A. Tsura[2], **E. A. Blinova**[1], A. E. Sizikov[1], V. A. Kozlov[1];*
[1]Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation, [2]Novosibirsk State Medical University, Novosibirsk, Russian Federation.

It is accumulated data about the lesion in apoptosis of peripheral blood mononuclear cells in patients with rheumatoid arthritis (RA). On this basis, we investigated the response of native T-cells (recipient-culture) in vitro under the transferring of the autologous apoptotic cultures (unstimulated, anti-CD3- (1mkg/ml) and dexamethasone- (1×10-4 M) stimulated donor-cultures under conditions of crowding and depleted medium).
The subject of the study were blood samples from patients with RA and healthy women, matched by age. Primary-induced apoptotic cultures (CFSE-) cultivated during 3 days, then their cellular and humoral components separately transferred to recipient-cultures, pre-stained with CFSE. After 4 days we obtained secondary-induced apoptotic cultures.
In norm, it was established the possibility to induce early apoptosis of T-cells by transferring the cellular and humoral components of the autologous apoptotic cultures. It was determined that under the equal conditions, transfer of stimulated by anti-CD3 antibodies cells significantly increased the sensitivity of T-cells to apoptosis, whereas stimulation by dexamethasone had a modulating effect.
In RA, it was demonstrated a marked level of early apoptosis of T-lymphocytes in primary-induced donor-culture, stimulated with anti-CD3 antibodies (CFSE-), and secondary-induced culture (CFSE+), compared both to the initial level of apoptosis and under transferring of unstimulated cells. Transfer of supernatants from autologous apoptotic anti-CD3- and dexamethasone-stimulated cultures obviously increased the levels of early apoptosis of T-cells in secondary-induced CFSE+ culture.
The obtained results indicated the features of induced early stage of apoptosis of T-cells by transferring of autologous apoptotic cultures in vitro in norm and in rheumatoid arthritis.

P.C2.05.02

Effects of homeostatic factors on the CD127 and CD215 expression by $CD4^+$ and $CD8^+$ T-cells in norm and rheumatoid arthritis

E. A. Blinova[1], *E. A. Pashkina*[1], *V. E. Balyasnikov*[2], *O. A. Chumasova*[1], *A. E. Sizikov*[1], *V. A. Kozlov*[1];
[1]*Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation,* [2]*Novosibirsk State Medical University, Novosibirsk, Russian Federation.*

It was formed assumption that homeostatic proliferation makes a contribution to the development and maintaining of autoimmune diseases, including rheumatoid arthritis (RA). In this study we investigated expression of α-chain of IL-7R and IL-15R on T-cells before and after culturing with homeostatic factors in norm and RA.
The study included 6 patients with RA and 6 healthy volunteers (average 61±4,5 and 59±4,8 years respectively). After isolation PBMCs were cultivated with or without IL-7 (50ng/ml), IL-15 (50ng/ml) and simultaneously IL-7+IL-15 during 7days. Activation with anti-CD3 antibodies (1mkg/ml) and IL-2 (100ME/ml) used as a positive control. Analysis of CD127 and CD215 expression was performed by flow cytometry.
$CD4^+$ and $CD8^+$ cells from RA patients demonstrated significantly low CD127 expression than donors' cells. There was no difference in CD215 expression and density of receptors to IL-7 and IL-15. Cultivation of PBMCs with or without stimulation led to a decreasing of CD127 expression. However, in patients' group it was observed the high density of CD127 on both T-cell populations under anti-CD3+IL-2 stimulation. Also it was an increasing in $CD4^+127^+$ cells under stimulation with IL-15 and $CD8^+127^+$ under stimulation with IL-7+15 compared to donors' values. There was a decreasing in a number of $CD4^+215^+$/$CD8^+215^+$ cells under stimulation with IL-15 and $CD8^+215^+$ cells – with IL-7 in RA compared to norm. aCD3 stimulation led to a high density of IL15R on $CD4^+$ cells from RA patients versus donors' values.
Obtained data indicated the changes in T-cell homeostasis and in responsiveness of T-cell subsets to homeostatic cytokines.

P.C2.05.03

Protein-engineered molecules carrying GAD65 epitopes and targeting CR1 selectively down-modulate disease-associated human B lymphocytes

I. Manoylov[1], ***G. Boneva***[1], *I. Doytchinova*[2], *N. Mihaylova*[1], *A. Tchorbanov*[1,3];
[1]*Laboratory of Experimental Immunology, Institute of Microbiology, Sofia, Bulgaria,* [2]*Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria,* [3]*National Institute of Immunology, Sofia, Bulgaria.*

Introduction: Type 1 diabetes mellitus is an autoimmune metabolic disorder characterized by chronic hyperglycemia, a presence of autoreactive T- and B-cells and autoantibodies against self-antigens. A membrane bound enzyme on the pancreatic beta-cells, GAD 65, is the main autoantigen in type 1 diabetes. Autoantibodies against GAD65 lead to beta-cells destruction and decline of pancreatic functions. The human complement receptor type 1 (CR1) on B- and T-lymphocytes has a suppressive activity on these cells. We hypothesized that it may be possible to eliminate GAD65-specific B cells from type 1 diabetes patients by using chimeric molecules, containing an anti-CR1 antibody, coupled to peptides resembling GAD65 B/T epitopes. These molecules are expected to bind selectively the anti-GAD65 specific B-cells by the co-crosslinking of the immunoglobulin receptor and CR1 and to deliver a suppressive signal. Materials and Methods: Two synthetic peptide epitopes derived from GAD65 protein, and anti-CD35 monoclonal antibody were used for the construction of two chimeras.
The immunomodulatory activity of the engineered antibodies was tested *in vitro* using PBMCs from diabetes patients. Results: A reduction in the number of anti-GAD65 IgG antibody-secreting plasma cells and increased percentage of apoptotic B-lymphocytes was observed after treatment of PBMCs from patients with type 1 diabetes with engineered antibodies. Conclusions: The constructed chimeric molecules are able to modulate selectively the activity of GAD65-specific B-lymphocytes and the production of anti-GAD65 IgG auto-antibodies by co-crosslinking of the inhibitory CR1 and the BCR. This treatment presents a possible way to alter the autoimmune nature of these cells.

P.C2.05.04

Interleukin-30 suppresses T cell activation in murine primary biliary cholangitis

H. Chen*, Y. Chuang;*
Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, Taipei, Taiwan.

Primary biliary cholangitis (PBC) is a chronic liver autoimmune disease. Our previous study found that PBC mice administered with adeno-associated virus-expressing IFN-γ (AAV-IFN-γ) showed a severe disease performance in early phase but subsequently leads to downregulation of chronic inflammation with an increase of Interleukin-30 (IL-30). IL-30, also called IL-27p28, has been shown to attenuate liver injury and fibrosis. In this study, we investigated whether IL-30 had an immunosuppressive function in PBC by administering mouse IL-30 expressing AAV (AAV-mIL-30) to 2-OA-OVA immunized PBC mice. At first, we defined the immunosuppressive function of AAV-mIL-30 in vivo by a well-known conA-induced hepatitis mouse model. The results showed that serum levels of IFN-γ and IL-12 were decreased in AAV-mIL-30 receiving conA induced hepatitis mice. In addition, the activation marker (CD25) of NKT cells and $CD4^+$ T cells in liver of AAV-mIL-30 receiving mice were also decreased. In PBC, the expression of CD25 and IFN-γ secretion in $CD4^+$ T cells were decreased in mice administered with AAV-mIL-30 three weeks post 2-OA-OVA immunization. Moreover, the frequency of $CD4^+CD25^{hi+}$ regulatory T cells was increased. These results suggested that IL-30 could suppress the activation and IFN-γ production of T cells and induce Tregs. Hence, IL-30 could be a novel therapeutic cytokine in PBC.

P.C2.05.05

PTPN22 negatively regulates immune complex-induced T cell proliferation by modulating dendritic cell antigen presentation and conjugate formation

F. Clarke*, H. Purvis, C. Sanchez-Blanco, E. Gutierrez Martinez, G. Cornish, P. Guermonprez, A. Cope;*
King's College London, London, United Kingdom.

The C1858T single nucleotide polymorphism in the hematopoietic tyrosine phosphatase *PTPN22* confers an enhanced susceptibility to multiple autoimmune diseases including rheumatoid arthritis and type 1 diabetes. Many of the associated autoimmune diseases have an autoantibody component to their pathology. Fc receptors (FcRs) recognise autoantibodies when they bind to autoantigens and form immune complexes. After immune complex binding and receptor crosslinking, FcRs signal via Src and Syk family kinases, leading to antigen uptake, presentation and cytokine secretion. PTPN22 negatively regulates Src and Syk family kinases proximal to immunoreceptor signalling cascades. We therefore hypothesised that PTPN22 regulates immune complex induced FcR responses in dendritic cells (DCs). Bone marrow derived DCs (BMDCs) from wild type and *Ptpn22*$^{-/-}$ mice were pulsed with ovalbumin:anti-ovalbumin immune complexes (ova ICs). Co-culture with WT $CD4^+$ OT-II T cells revealed that ova IC pulsed *Ptpn22*$^{-/-}$ BMDCs have an enhanced capability to induce T cell proliferation. This was associated with an increased capability of *Ptpn22*$^{-/-}$ BMDCs to present immune complex derived antigens and to form ova IC dependent DC-T cell conjugates. These findings highlight PTPN22 as a regulator of FcR mediated responses and provide a link between the association of $PTPN22^{R620W}$ with autoantibody associated autoimmune diseases.

P.C2.05.06

Circulating dendritic cells of patients with multiple sclerosis show inflammation-dependent gene expression changes following transmigration across an in vitro blood-brain barrier

M. De Laere[1], *S. Van Laere*[1], *J. Derdelinckx*[1], *B. Willekens*[2], *Z. Berneman*[1,2], ***N. Cools***[1];
[1]*University of Antwerp, Wilrijk, Belgium,* [2]*Antwerp University Hospital, Edegem, Belgium.*

Control of lymphocyte entry and migration into the brain is vital to regulate protective and pathological responses. Of interest is the finding that increased numbers of dendritic cells (DC) are present in the brain during neuroinflammation. Moreover, this has been associated with the regulation of local disease processes. But what prompts cells to enter the brain? In current study, we aim to delineate genetic differences between migrating and non-migrating DC using an in vitro model for the blood-brain barrier (BBB) and transcriptome profiling. Our findings demonstrate that circulating DC of patients with relapsing-remitting multiple sclerosis (RR-MS) and chronic progressive MS (CP-MS) show an increased expression of chemokine receptors CCR5 and CCR7. This finding is paralleled by augmented chemotaxis towards the respective chemokine ligands by DC from MS patients as compared to DC from healthy controls (HC). By means of an in vitro BBB model, 2 different subtypes of DC are isolated, namely migrating and non-migrating cells, of patients with MS and age- and gender matched HC. RNA sequencing and bioinformatics analyses show that gene expression of the 500 most variable genes differed between migratory and non-migratory cells when comparing RR-MS and CP-MS patients and HC. Using KEGG pathway analysis, the top gene pathways that were changed in migratory cells involved pathways associated with inflammatory processes, such as the Toll-like receptor and NOD-like receptor signaling pathway, and the Jak-STAT signaling pathway. Altogether, our observations contribute to current knowledge on the underlying pathological mechanisms and can provide interesting targets for therapeutic intervention.

P.C2.05.07

Mechanism of GP130 activation and regulation of its downstream signalling

F. Dehkhoda[1], *N. Durisic*[2], *Y. Chhabra*[1], *A. Brooks*[1];
[1]*The University of Queensland, Diamantina Institute, woolloongabba, Australia,* [2]*The University of Queensland, Queensland Brain Institute, St Lucia, Australia.*

GP130 is an archetypal member of the tall cytokine receptors and serves as a signal transducing subunit for IL-6, IL-11, IL-27, LIFR, and others. Physiologically, cytokines signalling via GP130 are involved in autoimmune and inflammatory diseases, including rheumatoid arthritis, inflammatory bowel diseases, and allergic asthma. Although numerous structural studies have elucidated dynamics of GP130 receptor complex, little is known about its activation and regulation of signal transduction. For this purpose, super resolution microscopy aided single particle tracking (PALM) of GP130 molecules was performed on non-stimulated and stimulated HEK293 cells to analyse diffusion properties of the receptors on the cell membrane. To analyse the signal transducing orientation of GP130, the extracellular domain of receptor was swapped with the leucine zipper dimerisation domain of c-jun transcription factor to generate GP130 dimers on the cell surface of pro-B cell line, Ba/F3.

The effect of length, charge, and rotation of the GP130 extracellular juxtamembrane region was investigated resulting in identification of active and inactive receptor configurations. Variable rotations of the GP130 transmembrane and intracellular domains induced by alanine insertions mimicking cytokine induced activation led to differential activation of JAK/STAT and MAPK signalling pathways and generated distinct proliferative responses in Ba/F3 cells. These models with FRET reporters fused after the JAK binding Box1-2 region were generated and movements of the intracellular domains of GP130 with associated JAK kinases were assessed. This is the first study aimed at determining the precise movements in GP130 receptor and will allow for a targeted drug design in near future.

P.C2.05.08

Up-regulation of EP2 and EP3 receptors in human tolerogenic dendritic cells boost the immunosuppressive activity of PGE_2.

G. Flórez-Grau[1]*, R. Cabezón*[2]*, K. E. Borgman*[3]*, C. España*[4]*, J. J. Lozano*[5]*, M. F. García-Parajo*[6]*, D. Benítez-Ribas*[4]*;*
[1]Department of Tumor Immunology, Radboud Institute for Molecular Life Sciences, Radboudumc, Nijmegen, Netherlands, [2]Institut d'investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, [3]Institut Ciencies fotoniques (ICFO), Castelldefels, Barcelona, Spain, [4]Dept of Immunology, Hospital Clinic de Barcelona,, Barcelona, Spain, [5]Centro de Investigación Biomédica en Red, Enfermedades Hepáticas y Digestivas (CIBERehd), Barcelona, Spain, [6]nsititució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

Dendritic cells (DCs) are essential in regulating both immunogenic and tolerogenic immune responses. PGE2, which is a lipid mediator produced during inflammation, has a key role i the of DCs maturation and, therefore, is vital in controlling the immune responses. The large variety of biologic functions governed by PGE2 are mediated by its signaling through 4 distinct E-type prostanoid (EP) receptors. Immunogenic DCs express EP2 and EP4, which mediate the PGE2 signaling. However, the presence and functionality of EP receptors in human tolerogenic DCs (tol-DCs), which have a suppressive phenotype, have been not assessed yet. To clarify the role of EP receptors in tol-DCs, we examined the expression of different EP receptors and their effect using selective agonists in human DCs. Our results shown that EP2 and EP3 expression are up-regulated in *in vitro*-generated tol-DCs compared with mature DCs (mDCs). Activation of EP2-EP4 has a direct effect on the surface expression of costimulatory molecules and maturation receptors, such as CD80, CD83, CD86, MHCII and CCR7 in tol-DCs, the latter being exclusively modulated by PGE2-EP4 signaling. Importantly, we find that EP2 and EP3 receptors are involved in tolerance induction through IL-10 production by tol-DCs. These results are in sharp contrast with the inflammatory role of EP4. Additionally, we observed that DCs generated in the presence of agonists for EP receptors, induce naive T cell differentiation toward polarized Th1/Th17 cells. Given the differential effects of EP receptors observed, EP receptor agonist/antagonists might become relevant novel drug templates to modulate immune response.

P.C2.05.09

Interleukin-2 is essential in long-term efficacy of regulatory T cells in type 1 diabetes

M. Gliwiński*, D. Iwaszkiewicz-Grzes, J. Sakowska, P. Trzonkowski;*
Department of Clinical Immunology and Transplantology, Gdansk, Poland.

Introduction: Type 1 diabetes (T1D) is a immunological disease which mainly affects children. Disease develops under the influence of autoreactive lymphocytes T that destroy insulin-producing β-cells. Transfer of autologous regulatory T cells (Tregs) delays the progression of the T1D. We decided to check the influence of interleukin-2 on Tregs therapy in clinical settings.
Materials and Methods: Regulatory T cells were isolated from the patients' peripheral blood with a GMP-compliant FACS-sorter. The expansion was performed under GMP conditions. Tregs were cultured for 8 days after the release of products to the clinic. Tregs and Teffs were cultured separately or co-cultured in a 1:1 ratio in the different concentrations of IL2. Survival of the cells was measured using 7-aminoactinomycin D staining. The levels of IL2 were also measured in sera of T1D patients treated with Tregs.
Results: We observed a rapid decrease in Treg cell survival without supplementation of IL2 in vitro. However, even low concentrations of IL2 were enough to limit cells death. Comparable concentrations of IL2 were found in vivo in the sera of T1D patients. In addition, simple co-culture of Tregs with Teffs without exogenous IL2 significantly improved the viability as Teffs consist a good source of endogenous IL2.
Conclusions: IL2 should be present in the culture constantly to keep Tregs viable. The levels of IL2 in vivo in T1D patients seem to be sufficient to protect Tregs transferred adoptively from cell death.
This work has been supported by National Centre for Research and Development, Poland: LIDER/160/L-6/14/NCBR/2015 and STRATEGMED1/233368/1/NCBR/2014.

P.C2.05.10

Exopolysaccharides produced by *Cyanobacterium aponinum* from the Blue Lagoon inhibit SYK and CLEC7a expression by dendritic cells and keratinocytes and dampen the function of T-cells

A. B. Gudmundsdottir[1,2]*, A. Brynjolfsdottir*[3]*, E. S. Olafsdottir*[4]*, I. Hardardottir*[1,2]*, J. Freysdottir*[1,2]*;*
[1]Landspitali - The National University Hospital of Iceland, Reykjavík, Iceland, [2]Faculty of Medicine, Biomedical Center, University of Iceland, Reykjavik, Iceland, [3]Blue Lagoon, Grindavik, Iceland, [4]Faculty of Pharmaceutical Sciences, University of Iceland, Reykjavík, Iceland.

Introduction: Regular bathing in the Blue Lagoon has beneficial effects on psoriasis. We have shown that exopolysaccharides (EPS-Ca) from one of the lagoon's dominating microbes, *Cyanobacterium aponinum*, increased dendritic cell (DC) secretion of IL-10 and DC induction of Tregs at the cost of the inflammatory Th17 cells. The objective of this study was to determine how EPS-Ca affects DCs and to determine its effects on stimulated T-cells and keratinocytes. Materials and methods: Human monocyte-derived DCs, $CD4^+$ T-cells and adult primary keratinocytes were stimulated with IL-1β, TNF-α and LPS; antibodies against CD3 and CD28; or TNF-α and either IFN-γ or IL-17, respectively, in the presence or absence of EPS-Ca. Cytokine secretion was measured by ELISA, expression of mRNA by rt-PCR and expression of surface molecules by flow cytometry. Results: EPS-Ca-treated DCs expressed more CD141 (linked to regulatory DCs) than DCs stimulated without EPS-Ca, and $CD141^+$ DCs secreted more IL-10 than $CD141^-$ DCs. EPS-Ca treatment decreased DC expression of Dectin-1 and transcription of key genes in the SYK-signaling pathway (CLEC7a and SYK). T-cells stimulated in the presence of EPS-Ca secreted less IL-10, IL-13 and IL-17 and expressed less CD69 than T-cells stimulated without EPS-Ca. EPS-Ca decreased keratinocyte secretion of CCL20 and CXCL10 and their transcription of SYK, CLEC7a, and psoriasis autoantigen CAMP (LL37). Conclusions: These results demonstrate inhibitory effects of EPS-Ca on important signaling pathways in DCs, T-cells and keratinocytes, all cells implicated in the pathogenesis of psoriasis, demonstrating the potential of EPS-Ca as a drug lead for the treatment of psoriasis.

P.C2.05.11

Interferon signature in patients with STAT1 gain-of-function mutation is epigenetically determined

E. Kaleviste[1]*, M. Saare*[1]*, R. Leahy*[2]*, W. Ip*[3]*, G. E. Davies*[3]*, P. Peterson*[1]*, K. Kisand*[1]*;*
[1]University of Tartu, Tartu, Estonia, [2]Our Ladys Childrens Hospital, Dublin, Ireland, [3]UCL Great Ormond Street Institute of Child Health, London, United Kingdom.

STAT1 is a transcription factor that mediates signals from interferons (IFNs). While STAT1 loss-of-function mutations confer susceptibility for viral and mycobacterial diseases the gain-of-function (GOF) variants of STAT1 lead to defective Th17 cell development and chronic mucocutaneous candidiasis (CMC). In addition, these patients develop autoimmune disorders: thyroid disease, cytopenias, type 1 diabetes, SLE, vitiligo, alopecia. STAT1 GOF mutation results in hyperphosporylation and delayed dephosphorylation of STAT1. However, the precise molecular mechanisms how this does affect intracellular signal transduction from cytokine receptors remains unknown.
We confirmed that after IFN-α stimulation the patients responded with exaggerated and prolonged upregulation of pSTAT1. However, there was no persistent induction of pSTAT1 ex vivo in spite of significantly upregulated IFN stimulated gene (ISG) expression in circulating blood cells in STAT1 GOF. Chromatin immunoprecipitation (ChIP) experiments showed that active chromatin mark H3K4me3 was significantly enriched in areas associated with ISGs and that STAT1 binding was enhanced in STAT1 GOF cells in comparison to normal cells. We found that STAT3 phosphorylation was not affected by STAT1 GOF after IL-21 stimulation but the balance of pSTAT3/pSTAT1 was significantly impaired.
In conclusion, autoimmunity in patients with STAT1 GOF mutation may be fostered by excessive effect of type I IFNs which is potentiated by epigenetic modifications. Impaired balance of pSTAT3/pSTAT1 after cell stimulation with STAT3-dependent cytokines may be responsible for defective Th17 cell development.

P.C2.05.12

The treatment outcomes in IgG4-related disease

F. Karim*, R. Bansie, S. Rombach, M. van Hagen, J. van Laar;*
Erasmus MC Rotterdam, Netherlands and Groene Hart ziekenhuis Gouda, Netherlands, Rotterdam, Netherlands.

Introduction: IgG4-related disease (IgG4-RD) is an emerging systemic inflammatory disease involving nearly all organs eventually leading to fibrosis. Prompt and adequate treatment to prevent irreversible organ damage is therefore pivotal. To evaluate the treatment outcomes, we studied a well-defined cohort of patients with IgG4-RD. **Method:** 32 patients with histologically confirmed IgG4-RD diagnosed between 1999 and April 2017 were included and reviewed for demographic and clinical characteristics. The response to treatment with glucocorticoids, disease modifying antirheumatic drugs, rituximab and other therapeutic interventions were evaluated. **Results:** Glucocorticoids as well as rituximab appeared successful therapeutic drugs leading to clinical remission (complete or partial remission) in all patients. Recurrences however, were frequently (62% versus 100%, respectively) seen. Diseases modifying antirheumatic drugs (DMARD's), including azathioprine, methotrexate and mycophenolate mofetil were effective in less than half of the cases. A minority of patients was treated with alternative treatments including hydroxychloroquine, thalidomide and infliximab which all appeared effective. Surgical intervention and radiotherapy in local disease seemed to induce clinical remission and were associated with low recurrence rates. **Conclusion:** Glucocorticoids and rituximab induce substantial responses as well as primary surgical intervention and radiotherapy, while the efficacy of DMARD's is limited. Based on the few data available, hydroxychloroquine, infliximab and thalidomide may be promising treatment options for second or third line strategies.

P.C2.05.13
OCA-B in the pathogenesis of Type 1 Diabetes

***H. Kim**, A. Shakya, C. German, D. Tantin;*
The University of Utah, Salt Lake City, United States.

Introduction: Type 1 diabetes (T1D) is an autoimmune disease caused by the destruction of insulin-producing beta cells in the pancreas. The mechanisms of T1D pathogenesis remain incompletely understood. Oct1 is a sequence-specific DNA binding transcription factor that in T cells potently regulates target gene expression with a co-factor, OCA-B. Both proteins control CD4+ T cell memory. Importantly, pathogenic CD8+ memory T cell frequency correlates with severity of insulitis in the non-obese-diabetic (NOD) mouse. The strongest OCA-B expression is found in pancreas-infiltrating-islet-reactive CD4+ T cells. Moreover, GWAS studies associate SNPs at multiple Oct1/OCA-B binding sites with T1D.
Materials and Methods: To study OCA-B effects on CD8+ T cell memory, WT or OCA-B KO naïve CD8+ T cells were cultured with a-CD3/28 for 2days. Subsequently, the cells were rested for 8 days without stimulus, and were re-stimulated for a recall response. To investigate impacts of OCA-B in the pathogenesis of T1D, NOD mice were injected with newly developed OCA-B inhibitor peptides or control.
Results: OCA-B loss in CD8+ T cells failed to induce recall responses in vitro. Strikingly, the inhibitor peptides reversed the elevated blood glucose in NOD mice. T cell infiltration and cytokine production in the pancreas was reduced compared to NOD mice treated with control peptide.
Conclusions: OCA-B regulates both CD4+ and CD8+ T cell memory and plays a role in T1D pathogenesis. To elucidate the mechanisms more deeply, OCA-B germline KO and T cell-specific OCA-B deleted NOD mice have been generated.

P.C2.05.14
Ethyl pyruvate treatment enhances regulatory T cell proliferation and function in type 1 diabetes

***I. Koprivica**, M. Vujičić, T. Saksida, D. Gajić, I. Stojanović;*
Institute for Biological Research "Siniša Stanković", Belgrade, Serbia.

Type 1 diabetes (T1D) is an autoimmune disease in which a strong inflammatory response causes insulin-producing pancreatic β-cell death. Ethyl pyruvate (EP), a stable pyruvate derivate, has exerted antioxidant and anti-inflammatory properties in several disease models. To test its therapeutic potential in T1D, EP was administered intraperitoneally to C57BL/6 mice with multiple low-dose streptozotocin (STZ)-induced T1D. EP treatment decreased T1D incidence and reduced the infiltration of cells into the pancreatic islets. *Ex vivo* analysis by flow cytometry showed that the EP treatment didn't change the number of immune cells in the spleen, pancreatic lymph nodes (PLN) or pancreatic mononuclear infiltrates (PMNI), nor the relative percentages of Th1, Th17 and Th2 cells. However, EP treatment increased the levels of regulatory T cells (Treg) in PLN and PMNI. After the EP treatment, all PLN Treg were $GITR^+CD127^-$, and an increase was noted in the percentage of $CD101^+$Treg, indicating a stronger suppressive activity. That was confirmed by an *in vitro* suppression assay, in which Treg from EP treated mice showed a higher capacity to supress effector T cell proliferation.
The number of $CXCR3^+$Treg and the presence of CD11a and CD62L per cell increased, which might imply an increase in Treg migration into the pancreas. However, a rise in the presence of $Ki67^+$Treg suggested that EP treatment also promotes Treg proliferation. These results show that EP treatment reduces T1D incidence in C57BL/6 mice by enhancing Treg proliferation, suppressive capacity, and recruitment into the pancreas. *This research is supported by MESTD, Republic of Serbia (#173013)*

P.C2.05.15
The role of IL-23TH17 pathway in Inflammatory bowel disease

***Y. Lakhoua Gorgi**[1], M. Majdoubi[1], J. Abdellatif[1], M. Jellouli[1], T. Dhaouadi[1], L. Mouelhi[2], T. Ben Abdallah[1], I. Sfar[1];*
[1]Research Laboratory in Immunology of Renal Transplantation and Immunopathology (LR03SP01), Tunis, Tunisia, [2]Department of Gastroenterology Charles Nicolle hospital, Tunis, Tunisia.

AIMS: Investigate a possible association between the functional polymorphisms: IL-17RC rs708567, IL-17F rs2397084 and IL-23R rs11209026 and the susceptibility to IBD and define the impact of these genetic variants on the IBD clinical forms **METHODS**: A cross-sectional case-control study involving 178 patients with IBD (108 CD and 70 UC) and 100 healthy control subjects was made. The molecular analysis was performed by PCR-RFLP. An ELISA tests were used to process to the quantitative determination of serum IL-17F and IL-23 cytokines.
RESULATS: Quantitatively, the mean level of IL-17F was significantly higher in patients than in controls (p = 0.003) and in CD patients with stricturing complications compared to other clinical forms (p = 0.006). The serum IL-23 levels were similar in patients and controls. The genotype G/G of IL-17RC polymorphism and the A/G genotype of IL-17-F SNP's seem to be associated to a higher occurrence of IBD and to the positivity of the ANCA antibodies in RCH patients in Tunisians (p = 0.049 and p = 0.008). For the R381Q IL-23R polymorphism, the frequency of the A allele was significantly higher in controls than in IBD patients (p <**0,0001)** suggesting a protective role of this SNP. **CONCLUSION:** The results of our study highlight the key role of IL-23Th17 pathway in the pathophysiological mechanisms of IBD. The prognostic interest of these markers deserved to be confirmed by further prospective studies on a larger cohort and by a functional analysis of IL-17F and IL-23mRNA expression

P.C2.05.16
Sulforaphane inhibits inflammatory responses of primary human T-cells by increasing ROS and depleting glutathione

***J. Liang**[1], B. Jahraus[1], E. Balta[1], J. Ziegler[1], K. Hübner[1], N. Blank[2], B. Niesler[3], G. Wabnitz[1], Y. Samstag[1];*
[1]Institute of Immunology, Heidelberg, Germany, [2]Department of Rheumatology, Heidelberg, Germany, [3]Department of Human Molecular Genetics, Heidelberg, Germany.

Sulforaphane (SFN), a compound in plants of the brassicaceae family, was reported to suppress cancer cell growth. Information about the relevance of SFN for human T-cells is limited. This is surprising as T-cells play a critical role in tumor control. We, therefore, investigated the effects of SFN on human untransformed peripheral blood T-cells. While SFN did not show cytotoxic effects and did not interfere with early T-cell activation, i.e. the formation of a mature immune synapse, it affected later activation events as upregulation of CD69 and CD25. The inhibitory effects of SFN could be rescued by thiol-containing antioxidants. In line with that finding, SFN led to an increase of intracellular reactive oxygen species (ROS) and a marked decrease of glutathione. Consistently, increased global cysteine sulfenylation was detected. Importantly, a major target for SFN-mediated protein oxidation was STAT-3, a transcription factor involved in the regulation of TH17-related genes. Moreover, costimulation-induced STAT-3 phosphorylation was significantly inhibited by SFN. In an unbiased gene expression signature analysis, we indeed found that TH17-related genes were predominantly inhibited by SFN. Since IL-17 and ROS regulation may be attractive targets for treating rheumatoid arthritis (RA), we tested the effect of SFN on whole blood from RA patients and found an increase in intracellular ROS levels in T-cells. Moreover, costimulation-induced expression of IL-17 was markedly decreased in SFN-treated T-cells from RA patients. Taken together, our study shows that SFN may act as a promising substance for therapeutic immune suppression via regulating the redox balance in human T-cells.

P.C2.05.17
Novel biomarkers of disease combined with ligand-directed targeted therapy for the control of autoimmune arthritis

*S. H. Venkatesha[1,2], S. Dudics[1,2], R. R. Meka[1,2], **K. D. Moudgil**[1,2];*
[1]University of Maryland School of Medicine, Baltimore, Baltimore, United States, [2]Baltimore Veterans Affairs Medical Center, Baltimore, United States.

Introduction: Rheumatoid arthritis (RA) affects millions of people worldwide. A key event here is the breakdown of self-tolerance leading to anti-self reactivity and tissue dysfunction/damage in the synovial joints. Current biomarkers for the diagnosis and prognosis of RA have inherent limitations. Similarly, the presently used drugs against RA are potent but their prolonged use is associated with severe adverse effects. Thus, there is a need for new biomarkers of disease and novel approaches to improve the therapeutic index of anti-arthritic drugs.
Materials and Methods: We addressed both these issues using the adjuvant-induced arthritis (AA) model of RA. We examined the micro-RNA (miRNA) expression profiles of the peripheral lymphoid cells of untreated/ treated arthritic and control rats, using miRNA-microarray. The data was subjected to statistical and bioinformatics analyses.
Results: We identified 8 specific miRNAs that were upregulated upon arthritis development. Collectively, these miRNAs targeted T cell response, angiogenesis, and bone remodeling pathways, but
individual miRNA specifically affecting Th17/Treg differentiation was also identified. Six of above 8 miRNAs were inhibited following arthritis treatment. For RA therapy, we employed a novel peptide
ligand that preferentially homes to inflamed joints after systemic administration, to direct drug-entrapping liposomes into the joints. Arthritic rats treated with these liposomes showed markedly
reduced severity of arthritis and decreased systemic toxicity compared to rats treated with free drug.
Conclusion: Our study has unraveled novel biomarkers of disease development and therapeutic response as well as an improved targeted therapy for autoimmune arthritis.
(Acknowledgement: grants from NIH and Veterans Affairs)

P.C2.05.18

miR-130a dysregulation contributes to CD1c$^+$ dendritic cell activation in Sjogren's Syndrome

A. P. Pinheiro Lopes[1,2], *J. A. van Roon*[1,2], *S. L. Blokland*[1,2], *M. Wang*[3], *E. Chouri*[1,2], *A. A. Kruize*[1], *B. M. Burgering*[3], *M. Rossato*[1,2], *T. R. Radstake*[1,2], *M. R. Hillen*[1,2];
[1]*Department of Rheumatology & Clinical Immunology, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands,* [2]*Laboratory of Translational Immunology, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands,* [3]*Center for Molecular Medicine, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands.*

Introduction. Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands and mucosal dryness. As dysregulation of CD1c-expressing dendritic cells (cDCs) might play a role in pSS pathogenesis, we investigated miRNA expression in isolated cDCs from pSS patients. ***Methods.*** cDCs were isolated from peripheral blood of two independent cohorts of patients and healthy controls (HC): a discovery cohort (15 pSS, 6 HC) and a validation cohort (14 pSS, 11 HC). qPCR-based profiling of 758 miRNAs was performed in the discovery cohort. A selection of 18 differentially expressed miRNAs was measured in the validation cohort using a custom array. To study miR-130a regulation, isolated cDCs from HC were stimulated with different TLR ligands. To discover novel targets of miR-130a, HEK-293T cells were transfected with a miR-130a mimic and protein synthesis was analysed using pulsed stable isotope labelling by amino acids in cell culture (pSILAC). ***Results.*** 36 miRNAs were downregulated in pSS patients versus HC in the discovery cohort. Of the 18 selected miRNAs for replication, the decreased expression of miR-130a and miR-708 was validated. cDC activation through TLR3 and TLR7/8 downregulated miR-130a. Transfecting HEK-293T cells with miR-130a identified downregulation of proteins involved in NF-kB signalling. ***Conclusions.*** We here show that miR-130a and miR-708 are decreased in pSS cDCs, which is induced by TLR-induced cell activation. In addition, we provide evidence showing that miR-130a targets mediators involved in NF-kB signalling and cDC activation at the protein level.

P.C2.05.19

Type 1 diabetes and related autoantibodies by the age of 13 years are not associated with probiotics administration in infancy

E. Savilahti[1], *M. Knip*[1], ***E. M. Savilahti***[1], *A. K. Kukkonen*[2], *M. Kuitunen*[1];
[1]*Children's Hospital, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland,* [2]*Skin and Allergy Hospital, University of Helsinki and Helsinki University Central Hospital, Helsinki,, Helsinki, Finland.*

Intestinal microbiota is thought to play a role in the development of type 1 diabetes (T1D), but prospective evidence on human studies is scarce. We used the data and material of a clinical double-blind randomized placebo-controlled trial on primary allergy prevention (n=1223) to investigate whether administration of a mix of pro- and prebiotics during late pregnancy and first 6 months of life was associated with prevalence of T1D during 13-year follow-up. Fourteen children had been diagnosed with T1D by November 2017, when the youngest participant was 13 years old. All those who developed T1D had complied to the intervention. Of them, 7 had received the active probiotic preparation. Factors known to have an effect on intestinal microbiota were similar among those developing diabetes and the remaining participants. In the 649 blood samples taken at the age 5, we analyzed GADA (96-585), IA-2A, IAA, ZnT8A 6.2 antibodies. Islet cell antibodies were measured only among those with positivity in any of the afore mentioned measurements. Levels above normal were found in altogether 25 children; 7 of them having increase in two or more measurements. Among these children, 12/25 with positive autoantibodies and 3/7 with ≥ 2 positive autoantibodies had received probiotic treatment during infancy. Probiotic or placebo treatment was thus not associated with positive autoantibodies at the age of 5 years. In conclusion, we found no effect of probiotic treatment in infancy on the development of T1D until the age of 13 years or on islet cell autoimmunity at age 5 years.

P.C2.05.20

Systemic level of IL-12p40 and its genetics polymorphisms in relapsing remitting multiple sclerosis

L. D. Miteva[1], *A. G. Trenova*[2], ***S. A. Stanilova***[3];
[1]*Trakia University, Medical Faculty, Stara Zagora, Bulgaria,* [2]*Medical University, Medical Faculty, Plovdiv, Bulgaria,* [3]*Trakia University, Medical faculty, Stara Zagora, Bulgaria.*

Introduction: Cytokines are tightly involved in the pathogenesis of multiple sclerosis as an inflammatory demyelinating disease. IL-12p70 and IL-23 share an IL-12p40 subunit. Two functional polymorphisms are identified within the IL12B gene encoding IL-12p40, rs17860508 and rs3212227. We aimed to determine the systemic IL-12p40 level among patients with relapsing-remitting multiple sclerosis (RRMS) and to explore the significance of both IL12B polymorphisms for RRMS risk. Materials and Methods: The genotyping was performed among 156 patients and 370 controls by PCR-based methods. Serum IL-12p40 concentrations were measured during remission of the disease by ELISA. Results: IL-12p40 serum levels were significantly higher in RRMS patients than in controls (184.66pg/ml; IQR:125-278.33 vs. 55.45pg/ml; IQR:40.95-72.3;p<0.0001). There was no statistically significant difference in serum IL-12p40 between patients without (EDSS≤1.5) or with mild/moderate (EDSS:2÷3.5) disability (p=0.821). The IL-12p40 levels decreased into the following order of genotypes of rs3212227: AA>AC>CC. Significantly lower IL-12p40 in patients carriers of CC-genotype (118.5pg/ml; IQR:85-161.67) compared to AA-genotype (198.14 pg/ml; IQR:156.67-298.6; p=0.0014) and AC-genotype (178.33pg/ml; IQR:125-278.33; p=0.024) was observed.
With regard to rs17860508, the genotype-22 and heterozygous-12 showed similar IL-12p40 quantities, slightly higher than genotype-11. There was a tendency for a higher frequency of AC/CC genotypes (48% vs. 43%; OR=1.227;95%CI:0.818-1.840) and combined AC/CC+12/22 (28% vs. 23%; OR=1.629;95%CI:0.720-3.719) in RRMS patients than controls, without reaching statistical significance. Conclusion: The significantly increased systemic IL-12p40 suggests the pivotal role of the IL-12 cytokine family in the pathogenesis of RRMS. Moreover, the functional effect of rs3212227 polymorphism was confirmed, although there is no association with RRMS risk.

P.C2.06 Immune signaling and therapy in autoimmunity - Part 6

P.C2.06.01

Therapeutic efficacy of systemic administration of orexin A in established experimental autoimmune encephalomyelitis

L. Becquet[1], *C. Abad*[1], *M. Leclercq*[1], *C. Miel*[1], *L. Jean*[1], *G. Riou*[1], *A. Couvineau*[2], *O. Boyer*[1,3], *Y. Tan*[1];
[1]*Normandie Univ, UNIROUEN, INSERM, U1234, PANTHER, Rouen, France,* [2]*Paris-Diderot University, INSERM U1149, Inflammation Research Center (CRI), DHU UNITY, Paris, France,* [3]*Normandie Univ, UNIROUEN, INSERM, U1234, Rouen University Hospital, Department of Immunology and Biotherapy, Rouen, France.*

Orexins (hypocretins, *Hcrt*) A and B are GPCR-binding hypothalamic neuropeptides known to regulate sleep/wake states and feeding behavior. A few studies have shown that orexin A exhibits anti-inflammatory and neuroprotective properties, suggesting that it might provide therapeutic effects in inflammatory and neurodegenerative diseases like multiple sclerosis (MS). MS is a highly prevalent autoimmune disease where encephalitogenic Th1 and Th17 cells trigger an inflammatory response in the CNS destroying the myelin sheath. Here, we investigated the effects of peripheral orexin A administration to mice with experimental autoimmune encephalomyelitis (EAE), a model of MS.
EAE was induced by active immunization of C57BL/6 mice with myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$ peptide. Orexin A was peripherally administered and the efficacy was assessed clinically, as well as by histopathology and cytokine and chemokine mRNA expression analysis in the CNS. Peripheral responses to MOG_{35-55} were studied by *ex vivo* antigen-recall assays.
Orexin A strongly ameliorated ongoing EAE, limiting the infiltration of immune cells and diminishing inflammation responses in the CNS. Moreover, orexin A treatment was neuroprotective by modulating glial cells and astrocytes. Despite its strong therapeutic effects, orexin A did not impair draining lymph node cell proliferation and Th1/Th17 cytokine production in response to MOG_{35-55} *in vitro*.
These results suggest that orexins may represent new therapeutic candidates that should be further investigated for MS treatment.
This work was supported in part by Inserm Transfert, and co-supported by European Union and Région Normandie. Europe gets involved in Normandie with European Regional Development Fund (ERDF).

P.C2.06.02

Targeting of tolerogenic dendritic cells towards heat-shock proteins: a novel therapeutic strategy for autoimmune diseases?

M. Jansen[1], *R. Spiering*[2], *P. van Kooten*[1], *I. Ludwig*[1], *W. van Eden*[1], *C. Hilkens*[2], ***F. Broere***[1];
[1]*Utrecht University, Utrecht, Netherlands,* [2]*Newcastle University, Newcastle upon Tyne, United Kingdom.*

Rheumatoid arthritis (RA) is an autoimmune disease caused by faulty regulation of the inflammatory process. A promising strategy to specifically target the pathogenic T cell response, while leaving other, protective T cell responses intact, is the use of tolerogenic dendritic cells (tolDCs). Since the self-antigen for RA is unknown and HSP70 has the potential to induce antigen specific Tregs, we investigate HSP70 as surrogate antigen. TolDCs were induced by dexamethasone and 1,25-dihydroxyvitaminD3 treatment of murine bone marrow derived dendritic cells (BMDCs). Subsequently, DCs were loaded with HSP70 peptide (B29) and co-cultured with mB29b TCR-Tg CD4 T cells. Furthermore, the effects of tolDCs on CD4$^+$ T cells *in vitro* and *in vivo* were investigated and tolDCs were injected in a proteoglycan induced arthritis mouse model. TolDCs exhibited a semi-mature phenotype and changed the phenotype of CD4$^+$ T cells to an immunomodulatory state, both *in vitro* and *in vivo*, when compared to mature BMDCs. T cells stimulated by tolDCs showed decreased proliferation and produced more IL-10. Interestingly, only mature tolDC significantly reduced arthritic symptoms, suggesting that a maturation stimulus is necessary to exert their function *in vivo*. These results indicate that cultured tolDCs are able to modulate the CD4$^+$ T cell response towards an anti-inflammatory state. Since the mice that were injected with matured tolDCs show reduced arthritis, we believe this mechanism might contribute to the efficacy of the tolDCs. Studying the immune modulatory capacity of tolDCs is essential for the further development of tolDCs for RA therapy.

P.C2.06.03

The immunomodulatory properties of the hookworm protein*Na*-AIP-1

***G. Buitrago**, S. Ryan, L. Jones, D. Pickering, P. Giacomin, A. Loukas;*
Australian Institute of Tropical Health and Medicine, James Cook University, Cairns, Australia.

Introduction: Inflammatory bowel disease (IBD) is an umbrella term for a group of immune-mediated conditions, which are characterised by idiopathic chronic inflammation of the gastrointestinal tract. Current treatment protocols for IBD are often poorly tolerated or ineffective. Multiple studies have described the potential efficacy of live parasitic helminth infection in alleviating symptoms of intestinal inflammation, in both human and animal models of disease. Consequently, there is interest in identifying potential immunomodulatory proteins secreted by helminths, which could be produced as a safer and more acceptable alternative to live worm therapy. Materials and Methods: We have identified, isolated and recombinantly expressed a novel candidate molecule from the secretions of the anthropophilic hookworm *Necator americanus* (*Na*-AIP-1). Results: Recombinant *Na*-AIP-1 was cloned and expressed using *Pichia pastoris* and purified via immobilised metal ion affinity chromatography. Intra-peritoneal treatment of mice with recombinant *Na*-AIP-1 ameliorated clinical, immunological and histological features of disease in two distinct murine models of colitis (TNBS and T cell transfer-induced colitis). Protection against colitis required the presence of CD11c+ dendritic cells and was associated with expansion of $CD4^+$ $Foxp3^+$ cells within mucosal tissue sites. Conclusions: Thus, *Na*-AIP-1 may represent an excellent candidate for further development as a novel treatment of autoimmune or inflammatory diseases of the intestine.

P.C2.06.04

IFN-alpha-treatment has different effects on NK cell populations in relapsing and monophasic rat experimental autoimmune uveitis

***M. Diedrichs-Möhring**[1], X. Liu[1,2], G. Wildner[1];*
[1]Section of Immunobiology, Dept. of Ophthalmology, University Hospital, LMU, Munich, Germany, [2]Present address: Ophthalmic Center of the Second Hospital, Jilin University, Changchun, China.

Interferon alpha (IFN-a) is a successful therapy of ocular Behçet's disease, but the underlying immune mechanisms are not yet fully understood. To further elucidate the therapeutic mechanisms we investigated the effect of IFN-a on leukocyte populations in two experimental rat models of relapsing and monophasic uveitis (EAU).
Uveitis was induced by immunization with either retinal S-Ag peptide PDSAg (monophasic/chronic EAU) or IRBP-peptide R14 (relapsing EAU). Recombinant human IFN-alpha was subcutaneously injected every day or every other day at different doses (1000 or 5000 IU) starting from different time points. Uveitis was graded clinically and histologically. Peritoneal exudate cells (PEC), lymph node (LN) cells and peripheral blood lymphocytes (PBL) were investigated for surface markers and cytokine expression by FACS-analysis. Preventive treatment with IFN-alpha starting with immunization significantly decreased the primary inflammation as well as relapses of R14-induced EAU, while PDSAg-induced, monophasic disease was deteriorated. Lymph node cells of PDSAg-immunized, IFN-a-treated rats revealed more TCR+ cells expressing IFN-γ, IL-17 or both, and less TCR+ cells producing IL-10 compared to the PBS-treated control group. IFN-a treatment decreased cell populations expressing CD161 in PDSAg-immunized Lewis rats, whereas in R14-induced EAU an increase in CD161+ cells.
IFN-a-treatment had different effects on relapsing and monophasic rat uveitis models. The deterioration of PDSAg-induced EAU by IFN-a-treatment might be due to an increase in leukocyte populations expressing inflammatory cytokines, and cells expressing CD161 might have a regulatory effect in R14-induced EAU.
Supported by Alexander von Humboldt/von Siemens -Foundation

P.C2.06.05

Immune modulating effects of low level laser therapy in oral lichen planus patients

***M. N. Draganova-Filipova**[1,2], M. Z. Mutafchieva[3], S. Y. Bachurska[4], G. T. Tomov[5], P. I. Zagorchev[6];*
[1]1. Department of Medical Biology, Faculty of Medicine, Medical University-Plovdiv, Plovdiv, Bulgaria, Plovdiv, Bulgaria, [2]Technological Center of Emergency Medicine, Plovdiv, Bulgaria, [3]2. Department of Periodontology and Oral diseases, Faculty of Dental Medicine, Medical University-Plovdiv,, Plovdiv, Bulgaria, [4]3. Department of of General and Clinical Pathology, Faculty of Medicine, Medical University-Plovdiv, Plovdiv, Bulgaria, Plovdiv, Bulgaria, [5]2. Department of Periodontology and Oral diseases, Faculty of Dental Medicine, Medical University-Plovdiv, Plovdiv, Bulgaria, Plovdiv, Bulgaria, [6]4. Department of Medical Physics and Biophysics, Faculty of Pharmacy, Medical University-Plovdiv, Plovdiv, Bulgaria, Plovdiv, Bulgaria.

Introduction: Oral lichen planus (OLP) is an autoinflammatory disease, result of activation of T-lymphocytes against unknown antigen. Therefore, destruction of the basal epithelial cells occurs and painful wounds are formed. The low-level laser therapy (LLLT) is considered as promising and harmless opportunity for OLP patients. The aim of this study was to compare the expression of pro- and anti-apoptotic markers in patient's biopsies and the salivary levels of Th-1, Th-2, Th-17-cytokines and SIgA before and after LLLT. Material and methods: 20 OLP patients and 20 healthy donors underwent LLLT with a diode laser (810nm), 3 times weekly for a month. The biopsies were taken before and after therapy and were analyzed immunohistochemically for the p53, p63, and bcl-2-expression. The reaction intensity was measured using a semiquantitative scale. The levels of IL1-β, IL-6, IL-17, and SIgA were measured by ELISA in unstimulated whole saliva.
Results: The results from immunohistochemistry showed that p53 and p63 expression was not changed ($p=0.69$ and $p=0.42$), but bcl-2-levels were increased ($p<0.02$) after therapy. In OLP patients cytokine levels of IL1-β (81±28pg/ml), IL-6 (26,9±10,3pg/ml) and IL-17 (19,0±9,7pg/ml) were higher compared to controls (21,2±3,7pg/ml; 14.1±6,2pg/ml and 11,9±5,1pg/ml) and decreased after therapy (66,2±31,7; 18.6±6,5pg/ml and 16,5±8,5pg/ml). SIgA in controls was 74,7±14,0μg/ml and respectively 88,1±26,0μg/ml and 92,2±23,7μg/ml before and after LLLT. Conclusions: LLLT is useful and harmless treatment modality for OLP patients, capable to balance the immune response. Keywords: immune regulation and therapy, inflammation, autoimmunity Acknowledgements: The investigation is sponsored by Grant HO-03/2014/MU-Plovdiv and partially by National *Science Fund*-Bulgaria-ДН-11/15/18.12.2017

P.C2.06.06

Critical role play by galectin-1 in the T cell mediated regulation of experimental colitis

***R. Fernandez**, O. Aberquilla, R. Sánchez, J. A. Bueren, M. Garin;*
Ciemat/IIS Fundación Jiménez Díaz/Ciberer, Madrid, Spain.

Foxp3-expressing regulatory T cells (Treg) are vital for maintaining balance among tolerance, adequate immune responses, and autoimmunity. In recent years, regulatory T cells are being developed as a cellular therapy with the potential to modulated unwanted immune responses. While their homeostasis has been extensively studied, their mechanisms of immunoregulation still remain to be fully clarified. We previously found that galectin-1 (GAL1), a β-galactoside-binding lectin, is expressed by human and mouse regulatory T cells and contributes to their immunosuppressive function *in vitro*. In this study, we examined the prophylactic and therapeutic effects of GAL1-deficient $CD4^+CD25^+Foxp3^+$ regulatory T cells (GAL1 KO-Tregs) on a T cell transfer *in vivo* model of inflammatory bowel disease (IBD). Our findings demonstrate that GAL1 KO-Tregs retained their capacity to modulate intestinal inflammation induced by $FOXP3^-$ $CD45Rb^+CD4^+$ naïve T cells into Rag-1 mice. However, the immunomodulation achieved was less efficient with respect to wild-type Tregs, as shown by the increase number of GAL1 KO-Tregs required for effective protection against IBD. In addition to this, the long term survival of the mice treated with GAL1 KO-Tregs was compromised when compared to those mice treated with wild-type Tregs. We conclude that galectin-1 plays a critical role in the T cell mediated regulation of experimental colitis and that an adequate expression of galectin-1 should be taken into consideration in future cell therapy protocols with regulatory T cells.

P.C2.06.07

Genetic microglia depletion ameliorates neuroinflammation in experimental autoimmune encephalomyelitis

***J. Han**, H. Lund, K. Zhu, M. Pieber, R. A. Harris, X. Zhang;*
Karolinska Institutet, Stockholm, Sweden.

Microglia are the principal resident immune cells in the central nervous system (CNS) and are believed to be versatile players in both inflammatory and physiological contexts. Although the dysfunction of microglia and microglia-induced neuroinflammation are implicated in the occurrence and progression of many neurological diseases, what remains largely enigmatic is the relative importance of microglia during different disease stages. We immunized $CX3CR1^{CreER}Rosa26^{DTA}$ (DTA) mice with myelin oligodendrocyte glycoprotein (MOG) to induce experimental autoimmune encephalomyelitis (EAE). Microglia depletion in DTA mice was initiated by injecting tamoxifen (TAM) for three consecutive days, resulting in almost complete loss of microglia after another 7 days. Depletion was initiated at day 5 of post-immunization during the period of disease initiation and early progression. The efficiency of microglia depletion was confirmed by using both microglia-specific P2ry12 immunohistochemistry and $CD11b^+CD45^+Ly6C^-Ly6G^-CX3CR1^+$ flow cytometry. The severity of EAE was evaluated using a clinical scoring system. At day 15 post-immunization, the peak of sickness, the mice were sacrificed and the tissues of brain and spinal cord were assessed by flow cytometry. Our preliminary results indicate that microglia depleted mice had less severe neuroinflammation as assessed by both the clinical scores and the data from flow cytometric analyses. Our results demonstrate that microglia are involved in the initiation and early progression of MOG-EAE. The tamoxifen-inducible $CX3CR1^{CreER}$ mice can be viewed as effective microglia-specific genetic tool to study the role of microglia in neurological diseases *in vivo*.

P.C2.06.08

Adipose-derived mesenchymal stem cells alter the Th17/Tregs axis in patients with rheumatoid arthritis yet not in patients with systemic sclerosis - preliminary data

***E. K. Kurteva**, G. Vassilev, M. Ivanova, E. Ivanova-Todorova, K. Tumangelova-Yuzeir, V. Boyadzhieva, N. Stoilov, R. Stoilov, D. Kyurkchiev;*
Medical University of Sofia, Sofia, Bulgaria.

Mesenchymal stem cells (MSCs) are fibroblast-like progenitor cells that possess the capacity to self-renew and can differentiate into several mesenchymal lineages. MSCs suppress the antigen-specific T-cell proliferation and cytotoxicity as well as inducing the generation of T reg cells. Regarding the pivotal role of the Th17/Treg axis in the pathogenesis of systemic sclerosis (SSc) and rheumatoid arthritis (RA), we aimed to study the immunosuppressive effects of adipose tissue-derived MSCs (AT-MSCs) conditioned medium on the percentage of Th17 and Tregs obtained from patients with SSc and RA. We enrolled 5 patients who fulfilled the 2013 ACR/EULAR Criteria for the classification of SSc and 13 patients matching the ACR/EULAR 2010 criteria for RA. AT-MSCs were isolated and cultured according to well established protocols. We used ELISA to quantitate the cytokines produced by AT-MSCs (IL-1, IL-10, IL-4, IFN-γ, IL-6, IL-8, CCL-5, RANTES, IL-17, TGF-β). Peripheral blood mononuclear cells (PBMCs) isolated from SSc and RA patients were cultured in AT-MSCs conditioned media as well as in control media. We performed flow cytometric analysis of the percentage of Th17 and T regs, and TGF-β levels produced by PBMCs were detected by ELISA.Our results demonstrated decreased percentage of Th17 cells (p=0.012) under the influence of AT-MSCs medium in RA yet not in SSc patients. The percentage of Tregs was raised only in RA patients: $CD4^+FoxP3^+$ lymphocytes (p=0,031), $CD4^+FoxP3^+CD25^{neg}$ (p=0.001). Further, in RA patients we found considerable increase in TGF-β levels (p=0.031) produced by PBMCs cultured with AT-MSCs medium as opposed to control PBMCs.

P.C2.06.09

$PD1^{hi}CXCR5^-$ peripheral helper T-cells producing IL21 and IFNγ are increased in systemic lupus erythematosus

***J. Land**, M. Parvaz, M. R. Ehrenstein;*
Centre for Rheumatology, Division of Medicine, University College London, London, United Kingdom.

Introduction: Follicular helper T (Tfh) cells are a specialised CXCR5+ T-cell subset that provide signals to B-cells, which are known to be increased in frequency in systemic lupus erythematosus (SLE) patients. Recently, a distinct circulating subset termed peripheral helper T (Tph) cells was described which are CXCR5- but have similar functional properties to Tfh.
Methods: Flow cytometry was performed on PBMC from 43 SLE patients and 12 healthy controls (HC) to determine the Tfh/Tph phenotype, including expression of PD1 and CXCR5. Production of IL21 and IFNγ was determined in 15 SLE and 5 HC.
Results: The percentage of circulating $CXCR5^+PD1^+$ Tfh cells was increased 1.5-fold in SLE compared to HC. The increase in $CXCR5^-PD1^{hi}$ Tph cell frequency was more pronounced being 4-fold higher in SLE (median 1.8%;range 0.3-9.6%) than in HC (0.4%;0.1-1.2%). SLE patients had an increased proportion of IL-21 producing Tfh and Tph cells. T-cells with high expression of PD1 demonstrated the largest proportion of $IL21^+$ and $IFN\gamma^+$ cells, regardless of CXCR5 expression. Double-positive $IL21^+IFN\gamma^+$ Tfh and Tph cells were also increased in SLE. The percentage of Tfh cells correlated with B-cell frequency in SLE (spearman's *r*=0.5;p=0.001) but not in HC, while Tph cell frequency negatively correlated with C3-levels (*r*=-0.361;p=0.031).
Conclusion: Tph as well as Tfh cells are increased in SLE. The increase in $IL-21^+$ T-cells within these two populations contained a large proportion of IFNγ producing cells. Tfh frequency did not correlate with disease activity, while Tph frequency correlated with C3 levels which are reduced in active disease.

P.C2.06.10

The role of the Tec kinase ITK and the transcription factor NFATc1 in disease pathogenesis of inflammatory bowel disease

***K. Lechner**, S. Mott, M. F. Neurath, B. Weigmann;*
Department of I., Medical Clinic, Erlangen, Germany.

Introduction: ITK, a member of the Tec family kinases, is expressed in T cells and involved in Th2-type mediated immune responses. Colitis patients can be successfully treated with CsA but CD patients not. Therefore, we started to investigate the role of ITK (interleukin-2-inducible T cell kinase) and the linked transcription factor NFATc1 in experimental colitis model.
Methods: Oxazolone colitis was induced in ITK deficient mice, conditional NFATc1-ΔCD4-KO mice and controls. Disease activity was measured by means of body weight, histological and endoscopic score of inflammation activity. Lamina propria mononuclear cells (LPMC) were isolated from these mice. The rate of apoptosis induction after treatment with CsA was assessed via flow cytometric analysis of AnnexinV/7AAD staining. Cytokine concentration was assessed using ELISA.
Results: In the oxazolone induced colitis model, ITK-KO mice are protected against the development of intestinal inflammation compared to control mice. Upon administration of CsA there is an induction of apoptosis in LPMCs from control mice. Conditional NFATc1-ΔCD4-KO mice show no protection against oxazolone induced colitis compared to control mice. Interestingly, administration of CsA could not prevent inflammation in these mice.
Discussion / Conclusion: Our results indicate that in the oxazolone induced colitis model, CsA induces enhanced apoptosis in LPMCs of control- and ITK-KO mice. NFATc1 doesn't seem to play a pivotal role in the development of intestinal inflammation since its knockout doesn't lead to protection against the induced colitis even after administration of CsA. Therefore, we suggest ITK to be a possible target for the therapy of colitis ulcerosa.

P.C2.06.12

Optimization of nanobodies for in vivo targeting of P2X7 ion channel on brain microglia and kidney immune cells.

***C. Pinto-Espinoza**[1], N. Schwarz[1], B. Rissiek[1], M. Junge[1], T. Magnus[1], F. Haag[1], C. Stortelers[2], F. Koch-Nolte[1];*
[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [2]Ablynx NV, Ghent, Belgium.

Nanobodies are the smallest antigen-binding domains derived from heavy chain antibodies, naturally occurring in Camelids.
The P2X7 ion channel is expressed by immune cells and promotes inflammatory responses. We generated nanobodies that effectively antagonize P2X7 and show benefit in animal models of inflammation (1). The clinical efficacy of these biologic antagonists is thought to reflect their excellent *in vivo* tissue penetration. However, little is known about the capacity of nanobodies to penetrate peripheral endothelial barriers or the highly restrictive blood-brain barrier (BBB) (2).
In this study, we reformatted P2X7 antagonistic nanobodies to improve tissue penetration. By genetic fusion, we dimerized them to increase avidity and added an albumin-specific nanobody to extend their *in vivo* half-life. By site-directed mutagenesis, we raised their isoelectric point (pI).
In vivo tissue penetration and efficacy of intravenously injected nanobodies at different doses and various time points were evaluated by determining the degree of P2X7 occupancy and P2X7 blockade using flow cytometry. The results show that very high nanobody doses were required to fully occupy and block P2X7 on microglia whereas a hundred-fold decrease in the dosage achieved the same effect on kidney resident immune cells, suggesting that high doses are required to allow complete translocation of nanobodies through the BBB.
(1) Danquah et al. (2016). Nanobodies that block gating of the P2X7 ion channel ameliorate inflammation. Sci Transl Med. 8:366-162.
(2) Li et al. (2016). Camelid single-domain antibodies: A versatile tool for in vivo imaging of extracellular and intracellular brain targets. J Control Release. 243:1-10.

P.C2.06.13

Exploring the immunosuppressive potential of venom-derived molecules

***R. Y. M. Ryan**[1,2,3], V. Lutzky[3], J. Potriquet[1], M. Ikonomopoulou[4], A. Lopez[2], J. J. Miles[1];*
[1]Australian Institute of Tropical Health and Medicine, Cairns, Australia, [2]Griffith University, Brisbane, Australia, [3]QIMR Berghofer, Brisbane, Australia, [4]IMDEA Food Institute, Madrid, Spain.

The unique combinations of potent, specific, and fast-acting molecules within venom act to rapidly disrupt vital biological processes in prey and predators. Ironically, the same characteristics that make venom biologically effective presents an ideal platform for the exploration of novel therapeutics and immunological pathways. This study mapped snake venom components with potent immune modulating abilities for drug development in the field of chronic inflammatory disease, of which many have no cure. Immunosuppressive venoms were fractionated using RP-HPLC and screened for activity against mitogen-induced activation. The effects of venom on human leukocytes were assessed using multiplex bead-based assays, flow cytometry, proliferation assays and cell viability assays. The results showed that specific venom fractions significantly inhibited IFNγ and TNFα release when primary leukocytes were stimulated with either Cell Stimulation Cocktail or CD3/CD28 activation beads. Interestingly, no change was observed in the myeloid compartment in response to lipopolysaccharide activation. Further, venom treatment reduced T-cell secreted cytokines without inhibiting cell proliferation or reducing cell viability, suggesting that these activation pathways are distinct in humans. Collectively, these data reveal novel venom-derived molecules specifically target and deactivate T-cells and could potentially be used to control or fine-tune the function of the human immune system.

P.C2.06.14

Proteomic analysis of a protective for rheumatoid arthritis TRAF-1 SNP.

A. Sarantopoulos[1], *K. Papavasileiou*[2], *G. Leonis*[3], *V. Melissas*[4], *I. Theodorou*[5], *P. Boura*[6], *A. Notopoulos*[7];
[1]*2nd Department of Internal Medicine, Hippokration General Hospital, Aristotle University of Thessaloniki, Greece, Thessaloniki, Greece,* [2]*Molecular Thermodynamics and Modeling of Materials Laboratory, Institute of Nanosciences and Nanotechnology, National Center for Scientific Research "Demokritos"., Athens, Greece,* [3]*Department of Chemistry and Department of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece,* [4]*Department of Chemistry, University of Ioannina, Ioannina, Greece,* [5]*UF d'Histocompatibilité et Immunogénétique chez Assistance publique- hopitaux de Paris. Faculty of Medicine UPMC Université Denis Diderot (Paris VII) / University Paris VII Paris 13, Paris, France,* [6]*Clinical Immunology Unit, 2nd Department of Internal Medicine, Hippokration General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece,* [7]*Department of Nuclear Medicine, Hippokration General Hospital, Thessaloniki, Greece.*

Introduction: Immunogenetic studies in Rheumatoid Arthritis (RA) have documented the positive correlation of various gene loci with incidence and/or disease profile. However, the description of gene loci negatively related to the incidence of RA is rarely documented. Even more seldom is the functional, proteomic analysis of such an SNP and of the respective intracellular signaling pathway. **Methodology - Results:** We performed the immunogenetic sequence of the seven exons of the gene TRAF1. 172 patients and 95 controls were genetically assessed. On the position 9:120905076 of exon 7, the registered polymorphism G/A (rs143265058) was described in the controls group. The same polymorphism was not confirmed in any of the patients. Further functional proteomic study of the polymorphism with computing programs (software), revealed that the presence of this polymorphism leads to a differentiation of the quaternary structure of TRAF1 protein. A further three-dimensional configuration of the TRAF-1 associated molecules was performed in order to assess the signaling deviation this SNP may elicit on the TNFα intracellular signaling pathway. **Conclusions:** The present reference is one of the extremely rare genetic studies describing a protective gene locus against rheumatoid arthritis, and a pioneer of its kind in the use of Applied Informatics in the depiction of the quaternary structure of the encoded protein. At the same time, it is one of the few immunogenetic studies describing the functional proteomics of the encoded protein, plotting on a molecular level specific interaction modifications affecting the intracellular signaling pathway of TNFα.

P.C2.06.15

Impact of rituximab therapy on IgG4 levels and B-cell phenotype in rheumatoid arthritis patients

A. Sarantopoulos[1], *E. Farmaki*[2], *M. Mytilinaiou*[3], *A. Gkantaras*[1], *A. Sarantopoulou*[2], *P. Boura*[3], *A. Tsirogianni*[4];
[1]*2nd Department of Internal Medicine, Hippokration General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece,* [2]*First Department of Pediatrics, Hippokration General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece,* [3]*Clinical Immunology Unit, 2nd Department of Internal Medicine, Hippokration General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece,* [4]*Department of Immunology and Histocompatibility, 'Evangelismos' General Hospital, Athens, Greece.*

Introduction: Rituximab is an effective therapeutic option in a variety of diseases. On IgG4-RD, apart from B-cell depletion, rituximab induces remission by reducing IgG4 levels. On RA, no study has inter-associated IgG4 levels and B-cell phenotype following rituximab therapy. We investigated whether B-cell depletion in RA: i) induces selective reduction of IgG4, ii) IgG4 alterations follow specific B-cell immunophenotype imprint. **Patients and Methods**: 31 RA patients, on a standard of care DMARD treatment and rituximab administration every 6 months for 2 years were investigated for alterations on disease activity along with IgG subclasses levels and B-cell immune profile. All parameters were assessed at enrollment (T0), and after 6, 12 and 24 months. On this 2-year period, all patients had been periodically receiving rituximab every 6 months. **Results:** After 2 years of rituximab administration, patients achieved a good response to treatment (EULAR criteria). Igs' levels were not statistically altered, though all of them declined. Furthermore, from IgG subclasses, only IgG4 levels statistically declined. Special features of emerging B-cell subpopulations have been identified in selected patients. **Conclusions:** This is the first time that IgG4 variations and B-cell emerging subpopulations are co-investigated in a non-IgG4RD after rituximab administration. Our results imply that IgG4 may be actively implicated in RA pathophysiology since disease remission is accompanied by only IgG4 level reduction among all classes and subclasses of Igs'. These findings open a new chapter in RA pathophysiology, indicating that this subclass is actively participating in the homeostasis of immune-mediated diseases other than IgG4-RD.

P.C2.06.16

Establishment of a chimeric human-mouse model of rheumatoid arthritis for pre-clinical testing

K. Schinnerling[1], *C. Schäfer*[1], *J. Maggi*[1], *C. Rosas*[2], *L. Soto*[3], *J. C. Aguillón*[1];
[1]*Universidad de Chile, Facultad de Medicina, Instituto de Ciencias Biomédicas, Santiago, Chile,* [2]*Universidad San Sebastián, Departamento de Ciencias Morfológicas, Facultad de Ciencia, Santiago, Chile,* [3]*Hospital Clínico Universidad de Chile, Sección de Reumatología, Santiago, Chile.*

Introduction: Preclinical testing of novel therapeutic approaches for rheumatoid arthritis (RA) requires a mouse model that imitates the human disease. To circumvent limitations due to differences between murine and human immune systems, we intended to establish a humanized mouse model of RA by transferring peripheral blood mononuclear cells (PBMCs) from healthy or RA subjects into immunodeficient mice. Methods: PBMCs were injected intravenously into 5-8-week old female NOD-*scid IL2rg*^{null} (NSG) mice. Human cell engraftment was monitored weekly and reactivity of human cells, recovered from spleens, towards synovial fluid (SF) and polyclonal stimulation, was assessed by flow cytometry. Human immunoglobulins (Ig) were quantified in serum by ELISA. Joint sections were stained with H&E. Results: Engraftment of human cells reached 36±5% in spleen, 77±4% in lymph nodes, 6±2% in bone marrow, and 22±4% in blood of NSG mice at six weeks after PBMC injection, regardless of whether PBMC from healthy or RA donors were used. The human graft consisted predominantly of activated CD4+ T cells which preserved their capacity to express IFN-γ in response to SF or polyclonal stimulation. Engrafted B cells continued producing IgM and IgG. Injection of RA patient-derived PBMC alone failed to generate histological alterations, however, in pilot experiments, additional intraarticular administration of SF, or injection of RA-derived CD4+ T cells together with SF-pulsed dendritic cells, resulted in cellular infiltrates and cartilage degradation. Conclusion: Although requiring further investigation, this chimeric human-mouse model of RA might provide an experimental system for preclinical testing of new therapies. Funded by FONDECYT3150453 and FONDECYT1140553.

P.C2.06.17

Characterization of MALT1 protease resistant CYLD knock-in and HOIL-1 knock-in mice

I. Skordos[1,2], *A. Demeyer*[1,2], *J. Staal*[1,2], *R. Beyaert*[1,2];
[1]*VIB-UGent Center for Inflammation Research, Ghent (zwijnaarde), Belgium,* [2]*Department of Biomedical Molecular Biology, Ghent University, Ghent (zwijnaarde), Belgium.*

Introduction: The paracaspase MALT1 is a key molecule in TCR induced signaling to NF-κB and steers TCR-induced gene expression by two distinct mechanisms: as a protease and as a scaffold protein. MALT1 proteolytic activity fine-tunes inflammatory gene expression and contributes to antigen-induced T cell proliferation as revealed by studies on MALT1 protease deficient (MALT1-PD) mice. Remarkably, MALT1-PD mice suffer from ataxia and lethal multi-organ inflammation, have reduced frequencies of thymic and peripheral regulatory T cells (tTregs and pTregs) and marginal zone (MZ) B cells, but have increased frequencies of effector T cells. Here, we investigated whether preventing the cleavage of an individual MALT1 substrate, CYLD or HOIL-1, leads to a similar phenotype. Materials and Methods: CYLD knock-in (KI) and HOIL-1 KI mice, expressing a non-cleavable form of CYLD or HOIL-1, were monitored for ataxia development and several organs were assessed for inflammation. The T cell activation status, the frequencies of tTregs, pTregs, MZ B cells, Th1, Th2, Th17, Tc1 and the $CD4^+$ T cell proliferation were determined by flow cytometry. Results and conclusions: In contrast to MALT1-PD mice, our KI mice did not develop ataxia or multi-organ inflammation. In addition, frequencies of tTreg, pTreg, MZ B cells, Th1, Th2, Th17 and Tc1 were comparable between KI and wild-type mice as were T cell activation status and $CD4^+$ T cell proliferation. To elucidate the potential function of CYLD and HOIL-1 cleavage by MALT1, we will conduct competitive bone marrow chimera studies and transcriptome analysis of TCR stimulated $CD4^+$ T cells.

P.C2.06.18

Phenotypic changes of lymphocyte populations in psoriasiform dermatitis animal model

M. I. Surcel[1,2], *R. Huică*[1,3], *A. Munteanu*[1], *G. Isvoranu*[1], *I. Pîrvu*[1], *D. Ciotaru*[1], *C. Constantin*[1,4], *O. Bratu*[3], *M. Neagu*[1,2,4], *C. Ursaciuc*[1];
[1]*Victor Babes National Institute of Pathology, Bucharest, Romania,* [2]*University of Bucharest, Faculty of Biology, Bucharest, Romania,* [3]*UMF „Carol Davila", Bucharest, Romania,* [4]*Colentina University Hospital, Bucharest, Romania.*

Psoriasis (PS), a T-cell mediated autoimmune disease, affects 3% of the population and the search for best immune markers in diagnosis and prognosis of this disease remains an unmet need. We have established a murine model of PS by topical application of Imiquimod (IMQ), and quantified the immune cell populations in both blood and spleen (study approved by the Ethical Committee).
Methods: C57BL/6 mice were constituted in 3 groups: 1) daily topical dose of IMQ (Aldara, Sweden) on the shaved back for 5 consecutive days; 2) control 1-daily topical with vaseline-based cream (Locobase, Netherland); 3) control 2 - no treatment. The progress and severity of inflammation (erythema, desquamation, induration) were scored daily. PASI score was calculated for each day. On the 6th day animals were weighted and euthanized. Spleen weight and bodyweight ratio assessed splenomegaly. Skin samples were histopathological examined. Lymphocytes were evaluated in peripheral blood and in spleen cellular suspension using flow-cytometry methods.
Results: Increase of erythema, scaling, thickening values and PASI score were registered; histology confirmed psoriasiform dermatitis. The main statistically significant changes in peripheral blood were T-CD4+ and B decrease correlated with T-CD8+ and NK increase. In spleen, B and all T cell populations decreased. No differences registered between control 1 and 2 groups.
Conclusions: Data suggest increased cytotoxicity in peripheral blood and decreased splenic cellularity as an expression of post-lesion skin inflammation. Immunophenotyping proves important to highlight the cytotoxic mechanism of disease.
Acknowledgement: Work supported by Core Program, implemented with support NASR, projects 18.21.02.01/18.21.02.02 and PN-III-P1-1.2-PCCDI-2017-0341/2018

P.C2.06.19

Bone marrow derived mesenchymal stromal cells suppress Th1 and enhance Th17 differentiation of antigen-activated CD4$^+$ T cells in vitro

J. L. Talbot[1], *L. S. Börnsen*[2], *M. R. von Essen*[1], *A. Fischer-Nielsen*[1], *R. S. Oliveri*[1], *M. Blinkenberg*[1], *F. Sellebjerg*[1];
[1]*Danish Multiple Sclerosis Center, København Ø, Denmark,* [2]*Danish Multiple Sclersosi Center, København Ø, Denmark.*

Mesenchymal stromal cells (MSCs) can modulate immune cell proliferation, activation and differentiation. The aim of this study was to assess how MSCs regulate the proliferation and differentiation of healthy immune cell responses to specific antigens known to induce different T helper cell (Th) responses.
Peripheral blood mononuclear cells (PBMCs) from healthy individuals (n=13) were incubated with bone-marrow derived MSCs from 1 MS patient (n=1) and 1 healthy donor (n=1). PBMCs were then stimulated with six different antigens for 7 days and analyzed by flowcytometry. The expression of the chemokine receptors CXCR3 and CCR6 were analyzed on $CFSE_{low}$ CD4$^+$ T-cells as surrogate markers for specific Th responses. Furthermore, we analyzed a panel of intracellular cytokines in CD8$^-$ T cells derived from healthy donors (n=7) after stimulation of PBMCs in coculture with MSCs.
Proliferation of CD4$^+$ T cells was significantly diminished when stimulated with antigens promoting Th1 responses. The expression of Th1- and Th17-associated chemokine receptor in CD4$^+$ T cell responses were significantly suppressed and enhanced, respectively, by mesenchymal stromal cells. Intracellular analysis revealed significantly increased expression of Th17 cytokines and decreased expression of Th1 cytokines on CD8$^-$ T cells cultured with bone marrow stromal cells.
Our results indicate that bone marrow stromal cell-induced suppression of CD4+ T cell proliferation may depend on the type of Th response. Stromal cells can suppress Th1-related responses and enhance Th17-related responses in CD4$^+$ T cells in a dose-response dependent manner.

P.C2.07 Immune signaling and therapy in autoimmunity - Part 7

P.C2.07.01

Anti-inflammatory activity of *Pistacia Lentiscus L*extracts in patients with IBD: an *in vitro* study

I. M. Boutemine[1], ***M. Amri***[1], *K. Layaida*[2], *L. Kecili*[2], *n. Kaddache*[2], *C. Fitting*[3], *J. M. Cavaillon*[3], *Z. C. Amir*[4], *S. Berkane*[2], *C. Touil-Boukoffa*[1];
[1]*University USTHB, Faculty of Biological Sciences, Laboratory of Cellular and Molecular Biology, Algiers, Algeria,* [2]*Gastroenterology department, Mustapha Pacha Hospital, Algiers, Algeria,* [3]*Cytokines & Inflammation, Pasteur Institute, Paris, France,* [4]*Pathology department, Mustapha Pacha Hospital, Algiers, Algeria.*

Introduction: Pistacia lentiscus L. (Anacardiaceae) (PL) is a flowering plant growing in Mediterranean area. It is traditionally used in the treatment of gastrointestinal upsets. Scientific findings also revealed the wide pharmacological activities from various parts of this species, such as antioxidant and anti-inflammatory activities.*Patients and methods:* Intestinal biopsies were performed during colonoscopy in patients with active-phase of inflammatory bowel diseases (IBD) (n = 27). They were cultured in Dulbecco's modified Eagle's medium at 37°C for 24 hours in the presence or absence of the aqueous extract (PLAE) (50 and 100 µg / ml) or fatty oil of PL (PLFO) (0,01% and 0,02% dissolved in 0,1% DMSO). In order to evaluate the effect of PL extracts on inflammation induced during IBD, pro-inflammatory and regulatory cytokines (IL-1β, IL-6, IL-8 and IL-10) as well as nitric oxide (NO) levels were evaluated in the culture supernatants. Moreover, microscopic observation and histological evaluation were performed to evaluate the effect of the PL extract on cell growth.*Results:* Our results demonstrate that the PL extracts significantly decrease the levels of pro-inflammatory cytokines (IL-1β, IL-6, and IL-8) and NO. However, these extracts increase the level of the immunoregulatory cytokine IL-10. Moreover, no cytotoxic effect was observed on the biopsies after cell culture.*Conclusion:* Our results suggest a beneficial effect of the *Pistacia lentiscus* extracts (aqueous extract and fatty oil) by reducing the inflammation induced during IBD. These findings indicate that PLFO and PLAE could constitute a new natural anti-inflammatory tool.

P.C2.07.02

Protective antibodies against rheumatoid arthritis

B. Xu[1], *B. Liang*[1], *D. Tong*[1], *E. Lönnblom*[1], *C. Ge*[1], *A. Olsson*[2], *C. Hofström*[2], *H. Lotsholm*[2], ***w. cai***[1], *R. Holmdahl*[1];
[1]*karolinska institutet, stockholm, Sweden,* [2]*Protein Expression and Characterization Drug Discovery and Development SciLife lab, stockholm, Sweden.*

Introduction: Rheumatoid arthritis (RA) is a common chronic inflammatory disease that affects mainly peripheral joints. Sadly here is no cure for RA yet. However, our earlier research found some of the defined epitopes located on Collagen II seem to be associated with protection against arthritis.
Materials and methods: Collagen antibody induced arthritis experiment: Arthritis is induced in 3-4 months old mice by intravenous injection of pathogenic monoclonal antibody (mAb) cocktail. One group received PBS and the others received antibodies to the certain epitopes respectively one day after injection of arthritogenic antibodies. All mice were boosted with 50ug Endotoxin (lipopolysaccharide) by intra-peritoneal injection on day 5. Arthritis was scored every second day until day 21 post immunization.
Results: Antibodies to this particular epitopes could suppress the development of arthritis if injected shortly after injection of arthritogenic antibodies
Conclusion: Antibodies to the certain epitope protects against arthritis in mice.

P.C2.07.03

The role of anticytokine autoantibodies in the pathogenesis of juvenile idiopathic arthritis

Y. Chen[1,2], *Y. Yang*[2], *B. Chiang*[2];
[1]*Department of pediatrics, Taipei City United Hospital, Taipei, Taiwan,* [2]*Department of pediatrics,, National Taiwan University Hospital, Taipei, Taiwan.*

Introduction: anti-cytokine autoantibodies (ACA) have been reported in healthy people and patients with a variety of autoimmune diseases, but their function is still debated. There is little study about ACA in juvenile idiopathic arthritis (JIA). The purpose of study is to examine anti-cytokine antibodies in the patients with juvenile idiopathic arthritis patients. **Material and methods:** Fifty- seven patients in whom oligoarticular, polyarticular and systemic type JIA were diagnosed before 16 years old were recruited on visits to the pediatric rheumatology department (from 2011 to 2015) and twenty healthy control were recruited. The ACA was assayed by indirect ELISA for anti-TNF alpha, anti IL-6 (inflammatory cytokine) antibodies. **Results:**Fifty-seven JIA patients and twenty healthy controls are recruited. Anti TNF alpha IgG and IgM show significantly higher level in oligoarticular and polyarticular subtypes than those of control group($p< 0.05$). Anti IL-6 IgG and IgM level showed significant higher in all subtype JIA group than control group ($P<0.01$). Anti IL-6 IgG showed higher level in active disease (CHAQ>0) than inactive disease(CHAQ=0) in oligoarticular and polyarticular suptypes. The anti IL-6 IgG level showed postivie correlation with WBC, PLT, ESR, CRP in systemic subtype. **Conclusions:**Anti-TNF alpha and anti IL-6 autoantibodies are higher in Juvenile idiopathic arthritis than those of healthy control in some suptypes. Although systemic type JIA is considered as autoinflammatory disease, we found autoantibodies in these patients. These antibodies might play a important role in pathologic mechanism, disease modulation or just reflect the increasing serum level of these cytokines.

P.C2.07.04

Protective effects of mixture of fifteen n-propyl polysulfides on ConA-induced hepatitis mediated by induction of regulatory macrophages

D. Djordjevic[1], *J. Milovanovic*[1], *M. Jurisevic*[1], *B. Stojanovic*[1], *O. Cvetkovic*[2], *M. Pergal*[2], *E. Ristic*[3], *D. Vojvodic*[3], *M. Simic*[4], *D. Manojlovic*[5], *M. Milovanovic*[1], *N. Arsenijevic*[1];
[1]*Faculty of Medical Sciences, Kragujevac, Serbia,* [2]*Institute of Chemistry, Technology and Metallurgy, Belgrade, Serbia,* [3]*Faculty of Medicine, Military Medical Academy, Belgrade, Serbia,* [4]*Center for New Technologies, Belgrade, Serbia,* [5]*Faculty of Chemistry, Belgrade, Serbia.*

Biologically active substances of garlic are different organosulfur compounds that exhibit potent antioxidant, anti-inflammatory, immunomodulatory and antitumor activities. The biological activity of organosulfur compounds is in direct correlation with the number of sulfur atoms. ConA induced hepatitis is acute form of inflammatory liver disease that shares some features of immune mediated liver diseases, including autoimmune hepatitis. In this study, immmunomodulatory and antiinflammatory potential of the mixture of fifteen n-propyl polysulfides was analyzed in ConA-induced hepatitis in mice. Mixture of fifteen n-propyl polysulfides was orally administered to C57BL/6 mice eight hours before intravenous injection of ConA. Disease severity was evaluated by liver enyzme assay, quantitative histology, mononuclear cell infiltration, cytokine production, liver endothelial cell activation and percentage of hepatocellular apoptosis. Mixture of fifteen n-propyl polysulfides almost completely prevented damage of liver tissue, attenuated production of inflammatory cytokines and enhanced infiltration of liver tissue with activated regulatory (CD86+CD206+IL-10+F4/80+) macrophages and regulatory T cells.
In conclusion, mixture of n-propyl polysulfides exerts highly antiinflammatory and immunomodulatory activity. This mixture enhances liver infiltration with regulatory macrophages and thus prevents ConA induced liver damage.

P.C2.07.05

Insufficient interleukin-10 production as a mechanism underlying pathogenesis of systemic juvenile idiopathic arthritis

M. Imbrechts[1], *A. Avau*[1], *J. Vandenhaute*[1], *B. Malengier-Devlies*[1], *K. Put*[1], *T. Mitera*[1], *N. Berghmans*[1], *O. Burton*[2], *A. Liston*[2], *L. de Somer*[3], *C. Wouters*[3], *P. Matthys*[1];
[1]*Rega Institute, KULeuven, Leuven, Belgium,* [2]*VIB-KULeuven, Leuven, Belgium,* [3]*University Hospital Leuven, Leuven, Belgium.*

Objective. Systemic juvenile idiopathic arthritis (sJIA) is a childhood immune-inflammatory disorder with unknown etiology. One of the concepts is that the disease results from an inappropriate control of immune responses to an initially harmless trigger. We investigated whether sJIA may be caused by defects in IL-10, a key cytokine in controlling inflammation.
Methods. IL-10 production was analyzed in a sJIA mouse model, which relies on injection of Complete Freund's Adjuvant (CFA) in IFN-γ deficient mice. Corresponding wild type (WT) mice develop a subtle and transient inflammatory reaction and were used to study the effect of IL10 neutralization. Cytokines and CRP were analyzed in plasma of sJIA patients (active: n=10; inactive: n=8) and healthy controls (n=15). Their PBMCs were used to study cell-specific defects in IL-10.

Results. Diseased IFN-γ deficient mice showed a defective IL-10 production in T_{reg} cells, B cells and NK cells, with B cells as the major source of IL-10. Neutralization of IL-10 in WT mice resulted in a chronic immune-inflammatory disorder clinically and hematologically reminiscent of sJIA. In sJIA patients, IL-10 plasma levels were strikingly low as compared to pro-inflammatory mediators. In addition, B cells from sJIA patients showed a decreased IL-10 production, both *ex vivo* and after stimulation.
Conclusion. Cell-specific IL-10 defects in sJIA mice and patients result in an insufficient IL10 production to counterbalance their pro-inflammatory cytokines. IL-10 neutralization in CFA-challenged WT mice converts a transient inflammatory reaction into a chronic disease and represents a model for sJIA in IFN-γ competent mice.

P.C2.07.06
Exploring the role of genetic variants in gene expression in the context of chronic inflammation

S. Koturan[1,2], *S. Menegatti*[1], *E. Latis*[1,2], *N. Rosine*[1,2], *H. Yahia*[1], *C. Miceli-Richard*[1,3,4], *E. Bianchi*[1,3], *L. Rogge*[1,3];
[1]*Institut Pasteur, Paris, France,* [2]*Université Paris Diderot, Ecole Doctorale BioSPC, Paris, France,* [3]*Immunological Research in Spondyloarthritis (IRIS), Unité Mixte de Recherche, Department of Immunology, Institut Pasteur, Paris, France,* [4]*Service de Rhumatologie, Hôpital Cochin, Paris, France.*

Introduction: Genome-wide association studies have provided detailed information about the genetic variants associated with chronic inflammatory diseases. However, for most genetic variants, the mechanism by which they act and the targeted cell populations are unknown. The general goal of this study is to decipher how SNPs associated with chronic inflammatory disease affect pathogenesis. In particular, we will determine which genetic variants affect cell type-specific gene expression using axial spondyloarthritis (SpA) as a model.
Materials and Methods: Whole blood samples from patients were cultured with a microbial stimulus (LPS) or a T-cell agonist (enterotoxin SEB). $CD14^+$ monocytes were isolated using magnetic bead selection from PBMCs followed by LPS stimulation. $CD4^+$ and $CD8^+$ T cell populations were isolated by cell sorting and stimulated with anti-CD3/CD28. We designed a gene expression panel that allows us to measure the expression of 755 genes that are either associated with chronic inflammatory diseases or the regulation of immune responses. Patients were genotyped using the Illumina Infinium® global screening array.
Results: Of the 755 genes analysed, around 600 genes were expressed in both LPS stimulated and SEB stimulated whole blood samples. For monocytes, we detected expression of 250 genes after stimulation. Our eQTL analysis revealed associations that regulate several immune-modulatory pathway-associated genes. The functional relevance of these findings warrants further study.
Conclusions: Our study demonstrates that the majority of genes genetically linked to chronic inflammation are expressed in whole blood and immune cell populations from SpA patients pointing to new insights in pathogenesis of chronic inflammatory diseases.

P.C2.07.07
Comparative analysis between the *in vivo* biodistribution and therapeutic efficacy of adipose-derived mesenchymal stromal cells administered intranodally in experimental colitis

M. Lopez-Santalla[1], *P. Mancheño-Corvo*[2], *A. Escolano*[3], *R. Menta*[2], *O. Delarosa*[2], *J. Abad*[4], *D. Büscher*[5], *J. M. Redondo*[6], *J. A. Bueren*[1], *W. Dalemans*[7], *E. Lombardo*[2], *M. Garin*[1];
[1]*CIEMAT/IIS Fundación Jiménez Díaz/CIBERER, Madrid, Spain,* [2]*Tigenix SAU, Madrid, Spain,* [3]*The Rockefeller University, New York City, United States,* [4]*Coretherapix, Madrid, Spain,* [5]*Grifols, Barcelona, Spain,* [6]*Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain,* [7]*Tigenix NV, Leuven, Belgium.*

INTRODUCTION: Mesenchymal stem cells (MSCs) have immunomodulatory properties allowing their use for treatment of a wide variety of immunological disorders. The mechanisms that are involved in their therapeutic effects have not been fully determined. Immunomodulatory effect of MSCs takes place both by direct cell-to-cell contact and by soluble factors. A small proportion of infused MSCs can traffic to the draining lymph nodes accompanied with an increase of different types of regulatory immune cells. Intranodal injection of cells is being used in the clinic for treatment of cancer and allergy and can be an alternative route for MSC administration.
METHODS: We analyzed the biodistribution and the efficacy of $Luciferase^+$ adipose-derived MSCs (Luci-eASCs), infused through the inguinal LNs (iLNs), in an experimental mouse model of TNBS-induced colitis.
RESULTS: Luci-eASCs were able to modulate the acute inflammatory response induced by the administration of TNBS. The large majority of Luci-eASCs were found in the iLNs and in the adipose tissue surrounding the injection site. Increase bioluminescence signal was found in the intestine of colitic mice compared to healthy controls. Luci-eASC-infused mice were stratified according to their positive response to the Luci-eASC treatment. A higher accumulation of Luci-eASCs was found in popliteal, parathymic, parathyroid and mesenteric LNs in those mice that had a positive response to Luci-eASCs.
CONCLUSIONS: Acute intestinal inflammatory responses can be modulated by intranodal administration of Luci-eASCs. The accumulation of the eASCs at the inflamed intestine might be beneficial to achieve an optimal modulation of inflammation following intranodal administration.

P.C2.07.08
***In vivo* biodistribution of adipose-derived mesenchymal stromal cells administered intraperitoneally in experimental colitis**

M. Lopez-Santalla[1], *P. Mancheño-Corvo*[2], *A. Escolano*[3], *R. Menta*[2], *O. Delarosa*[2], *J. M. Redondo*[4], *J. A. Bueren*[1], *W. Dalemans*[5], *E. Lombardo*[2], *M. Garin*[1];
[1]*CIEMAT/IIS Fundación Jiménez Díaz/CIBERER, Madrid, Spain,* [2]*Tigenix SAU, Madrid, Spain,* [3]*The Rockefeller University, New York City, United States,* [4]*Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain,* [5]*Tigenix NV, Leuven, Belgium.*

INTRODUCTION: The use of mesenchymal stem cells (MSCs) in the clinical field has gathered tremendous momentum over the last decade due to the varying levels of success in treatment of inflammatory and autoimmune diseases thanks to their immunomodulatory properties. The immunomodulatory effect of MSCs takes place both by direct cell-to-cell contact and by soluble factors. Although the biodistribution of MSCs highly depends on the route of administration, similar efficacy has been described regardless the administration route used. Several studies have shown that a small proportion of the infused MSCs can be found at the site of inflammation. At present, it is unknown whether the migration of the MSCs to the inflamed tissues is a prerequisite to achieve their beneficial effect.
METHODS: We analysed the biodistribution of intraperitoneally (IP) administered luciferase-expressing human expanded adipose derived stem cells (Luci-eASCs), in a mouse model of colitis. Stratification of mouse responses to the Luci-eASC treatment allows us to define whether a correlation between the *in vivo* biodistribution of Luci-eASCs and their therapeutic efficacy exists.
RESULTS: IP administered Luci-eASCs were mainly found in the liver, spleen and intestine. In the intestine of colitic mice, a higher accumulation of Luci-eASCs was found, compared to healthy controls. The bioluminescence signal in the intestine tended to increase at the expense of a decrease in the liver in the `responder´ mice.
CONCLUSIONS: These data thus suggest that the accumulation of the eASCs to the inflamed tissues is beneficial in order to achieve modulation of the inflammatory insult.

P.C2.07.09
Peptide-specificity of the CD4 T-cell response to immunogenic therapeutic antibodies in healthy donors and in patients.

S. Meunier, M. de Bourayne, M. Hamze, ***B. Maillere****;*
CEA, Gif sur Yvette, France.

Immunogenicity of therapeutic antibodies remains an important limitation to their clinical use. Infliximab (Ifx), Rituximab (Rtx), Adalimumab (Adm) and Natalizumab (Ntz) are all known to induce neutralizing anti-drug antibodies (ADA) in many patients. Because CD4 T-cells initiate immune responses, we identified the CD4 T-cell epitopes of these antibodies. With the perspective to anticipate immunogenicity of therapeutic antibodies, the T-cell epitope mapping was carried out using cells collected in healthy donors. CD4 T-cells were expanded by several weekly rounds of antigen-specific stimulation and the T-cell specificity was assessed by ELISPOT using overlapping peptides. Nine epitopes were identified in the VL and VH chains of chimeric Rtx and Ifx and overlapped CDR or FR regions. Nine and eleven T-cell epitopes were found in the humanized and human antibodies Ntz and Adm, respectively. Several peptides were common to multiple donors and their location appeared to rely on their affinity for HLA molecules and their content in mutations with respect human germline sequences. T-cell reactivity of the identified peptides was evaluated in patients having developed (ADA+) or not developed ADA (ADA-). Two third of the T-cell epitopes of Rtx and Ifx identified from the healthy donors stimulated PBMCs from ADA+ patients, while T cell reactivity of Adm T-cell epitopes Is higher in ADA+ patients than in ADA-. T-cell epitopes promote the secretion of a diversity of cytokines either favoring or reducing ADA production. Together our data provide new insights on the origin and mechanisms of immunogenicity of therapeutic antibodies.

P.C2.07.10
A CD4 T cell repertoire specific to immunogenic self therapeutic proteins exists in healthy donors before any injection

S. Meunier, A. Azam, M. de Bourayne, ***B. Maillere****;*
CEA, Gif sur Yvette, France.

Many hormones, cytokines and clotting factors are used as therapeutic molecules but many of them produce anti-drug antibodies (ADA), although their peptide sequence is of human origin. To quantify the rare antigen-specific T-cells from healthy donors, who have never been exposed to the antigen, CD4 T-cells were stimulated by weekly rounds of stimulation by antigen-loaded dendritic cells and their specificity was assessed by IFN-γ ELISpot. The number of specific T-cell lines was used to estimate the frequency of circulating T-cells. Both forms of IFN-β induced ADA in multiple sclerosis patients. We identified a CD4 T-cell repertoire specific to IFN-β and demonstrated the T cell epitopes spread all over the IFN-β sequence, two regions being common to multiple donors. H2-relaxin is a hormone with a insulin-like structure and has been found to induce ADA in scleroderma patients.

A very large repertoire of T cells specific for H2-relaxin was found in the healthy donors. We also identified two major T-cell epitopes hosted in the α and β chains and common to multiple donors. The frequency of FVIII-specific CD4 T cells in healthy donors was very high and similar to that of T cells specific for foreign antigens. We also observed that FVIII-specific T cells originated from both the naïve and central memory cells. Altogether our results showed that endogenous expression of IFN-β FVIII and H2-relaxin is not sufficient to prevent an escape of CD4 T cells from negative thymic selection and to abrogate immunogenicity of the recombinant forms.

P.C2.07.11

IgG and IgM play a main role in the development of demyelinating lesions and axonal damage in patients with multiple sclerosis.

Ú. Muñoz[1], C. Sebal[1], E. Escudero[1], M. M. Esiri[2,3], I. Iturrieta[1], C. Sloan[2], A. Jayo[1], M. C. Sádaba[1];
[1]Facultad de Medicina, Instituto de Medicina Molecular Aplicada (INMA), Universidad San Pablo-CEU, CEU Universities., Madrid, Spain, [2]Nuffield Department of Clinical Neuroscience, University of Oxford, Headington, Oxford, United Kingdom, [3]The Oxford University Hospitals NHS Trust. Headley Way, Headington, Oxford, UK., Oxford, United Kingdom.

Introduction. Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system characterized by demyelination and axonal damage. We have described that IgG and IgM co-localized with complement and macrophages on oligodendrocytes and axons. Oxidative stress is also a hallmark of the lesions. Therefore, we aimed to analyze the role of antibodies and oxidative stress in these patients.
Materials and Methods: Brain samples from patients suffering from MS, neurodegenerative diseases (Alzheimer, Parkinson, Frontotemporal Dementia (FTD), amyotrophic lateral sclerosis (ALS)), and control group. Immunohistochemistry and immunofluorescence techniques were used to detect IgG, IgM, oxidative stress (EO6), axonal damage (amyloid precursor protein, APP). TUNEL assay was used to detect cell damage.
Results. EO6, IgG and IgM were not detected in samples from patients with Alzheimer, Parkinson or control group. In samples from MS patients, IgG and IgM were observed on TUNEL positive oligodendrocytes located in normal appearing white matter and IgM, were observed on APP positive axons in demyelinated lesions. EO6 positive axons were observed in samples from FTD and ALS patients. Lipid oxidation colocalized with APP in this individuals.
Conclusions. IgG and IgM play a main role in the development of demyelinated areas and IgM in axonal damage in MS patients. For the first time, oxidative stress were detected in FTD and ALS patients, which is associated with axonal damage. Our work draw attention in the different pathologic mechanism involved in neurodegenerative diseases.
Acknowledgments: Universidad San Pablo CEU and Banco Santander for grants PI14/01620, USP-BS-PPC16/2012 and MEMERG-1,

P.C2.07.12

TNF hampers intestinal tissue repair in colitis by restricting IL-22 bioavailability

***J. Ninnemann**[1], C. Winsauer[1], A. Kühl[2], S. Nedospasov[3], A. Kruglov[1];*
[1]Deutsches Rheuma Forschungszentrum, Berlin, Germany, [2]Charite Medical School, Berlin, Germany, [3]M.V. Lomonosov Moscow State University, Moscow, Russian Federation.

Successful treatment of chronic inflammatory diseases integrates both cessation of inflammation and induction of adequate tissue repair processes. One of the examples of such therapy is Tumor Necrosis Factor (TNF) inhibition in IBD patients. However, molecular mechanisms of intestinal repair upon TNF blockade during IBD remain not understood. Here, by the usage of human TNF Knock-in mice (hTNFKI) in a model of adoptive T cell transfer, we revealed that TNF interferes with tissue repair program via induction of soluble natural antagonist of IL-22 (IL-22Ra2; IL-22BP) in the colon and abrogates IL-22, STAT3-mediated mucosal repair during colitis. Pharmacological T-TNF blockade reduced IL-22BP expression in the colon leading to the increased IL-22 levels, colonic epithelial cell proliferation and restoration of colonic epithelium functions. Thus, our data revealed the mechanism of how anti-TNF therapy induces mucosal healing and provides novel potential targets for IBD treatment in humans. Work was supported by SFB633 and NE1466/2-3 grant from DFG (S.N.), and Russian Science Foundation grant #14-50-00060 (S.N.) and # 17-74-20059 (A.K.).

P.C2.07.13

MCC950 attenuates colonic inflammation in spontaneous colitis mice

***A. P. Perera**, R. Eri;*
Shool of Health Sciences, Launceston, Australia.

MCC950 is a potent, highly specific small molecule inhibitor of both canonical and noncanonical activation of NLRP3 inflammasome and has been evaluated in a diverse array of NLRP3 engaged inflammatory diseases. However, the effect of MCC950 on colitis has not yet been reported. In the present study we investigated the effect of MCC950 in a spontaneous chronic colitis mouse model Winnie, which mimics human ulcerative colitis. Oral administration of 40 mg/kg MCC950 for three weeks at chronic stage of colitis significantly ameliorated colitis with improved body weight gain, colon length, ratio of colon weight to body weight, disease activity index and histopathological scores of MCC950 treated Winnie mice were significantly reduced suggesting not only attenuation of ongoing colitis but also delay of disease onset. MCC950 significantly suppressed IL-1β and IL-18 cytokine expression at both mRNA and protein levels in Winnie colons.
Additionally, MCC950 also effectively suppressed the release of proinflammatory cytokines (IL1-α, IL17, TNF-α and IFNγ) and chemokine (MIP1a) in mucosal explants. Moreover, MCC950 treatment resulted in a significant decrease of IL-1β release and activation of caspase-1 in Winnie explants and *in vitro* macrophage cells isolated from Winnie mice. The treatment of 10μM MCC950 in Winnie mucosal explants shows, for the first time, the contribution of anti-inflammatory effects resulting exclusively from inhibition of canonical and non-canonical NLRP3 inflammasome activation in colitis. Taken together, our results illustrate the efficacy of MCC950 in the treatment of murine ulcerative colitis and provides avenue for a novel therapeutic agent for human inflammatory bowel diseases.

P.C2.07.14

Effect of Tripterygium wilfordii polycoride on inflammation and TLR4 signaling pathway in ulcerative colitis rats model

***D. Qin**[1], Y. Zhou[2], Q. Yang[1], C. Zhang[1], S. Zhang[1], Q. Dai[1], X. Yang[1];*
[1]The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, China, [2]The First Clinical Medical College of Zhejiang Chinese Medical University, Hangzhou, China.

Tripterygium wilfordii polycoride (TWP) is a water-chloroform extract from a kind of Chinese medicine-*Tripterygium wilfordii* Hook. f. It preserves a good immune regulation effect and has been applied to the treatment of some autoimmune diseases. However, no related studies on its role in ulcerative colitis (UC) were reported till now. In this work, we investigated the effect of TWP on UC inflammation and toll-like receptor 4 (TLR4) signaling pathway in TNBS-alcoholic UC rats model. The study found that TWP can significantly improve the symptoms of UC rats model and has a good inhibitory effect on intestinal inflammation. It not only promotes the healing of mucosal defects, but also inhibits the infiltration of inflammatory cells in mucosal lesions to acquire mucosal healing. Whereas, these effects are dose-dependent. On the research of its mechanism of action, we found that TWP exerts anti-inflammatory effects through inhibiting the protein expression of TLR4/MyD88-dependent and non-dependent signaling pathways, thereby inhibiting the release of downstream inflammatory factors.

P.C2.07.15

Clinical study on the effect of tripterygium wilfordii polyglycoside on intestinal inflammation in inflammatory bowel disease

***D. Qin**[1], Y. Wang[1], G. Ni[1], C. Zhang[1], Y. Mao[1], G. Fang[1], L. Xu[1], X. Hu[1], Q. Qiao[1], S. Zhu[1], Q. Yang[2], G. Cen[2];*
[1]The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, China, [2]The First Clinical Medical College of Zhejiang Chinese Medical University, Hangzhou, China.

Objective: To observe the effect of TWP on intestinal inflammation in IBD patients. Method: 32 IBD patients, with steroid hormones resistance or dependence or taking other immunosuppressive agents with intolerable side effects, were selected. We give TWP oral treatment (60mg/d) on the basis of the original aminosalicylic acid treatment (4g/d),after treated for 24 months, we make a before and after treatment comparison analysis for patients, including clinical manifestations, endoscopic mucosal lesions, tissue inflammation, blood changes, mucosal healing, immune response and toxic side effects. Result:(1) TWP improve the symptoms of IBD patients significantly ;(2)The scores of endoscopic MES and SES-CD were significantly decreased after TWP treatment;(3)After treatment, the score of mucosal inflammation injury in IBD patients is decreased; (4) The levels of ESR, CRP, PLT,HGB,ALB are improved;(5)25 patients have a complete response to TWP after treatment, partial response are 7 cases;(6) Hormone dependents included in this study is successfully discontinued after taking TWP 12 weeks and have a complete response to TWP treatment;(7) The major toxic side effects include gonadal suppression,mild bone marrow suppression and mild liver damage in the study, but they will turn back to normal after reducing the dosage of TWP.Conclusion: TWP can relieve the clinical manifestation of IBD, inhibit intestinal inflammatory activity and acquire mucosal healing. Meanwhile, TWP can help the steroid hormone dependent patients to reduce the dosage of hormone smoothly until discontinuation, and to get the response and remission to the treatment.

P.C2.07.16

Immunogenicity assessment of three anti-TNFα blockers (Infliximab, Adalimumab and Etanercept) in 42 chronic inflammatory disease affected patients.

S. Salah[1], *I. Mezghiche*[1], *M. Benidir*[1], *M. Djennane*[2], *D. Fodil*[3], *G. Hamadi*[1], *N. Zaabat*[1], *S. Tefiani-Lefkir*[3], *H. Amroun*[4], *N. Attal*[1];
[1]*Pasteur Institute of Algeria, Algiers, Algeria,* [2]*Tizi Ouzou Hospital, Tizi Ouzou, Algeria,* [3]*Beni Messous Hospital, Algiers, Algeria,* [4]*Parnet Hospital, Algiers, Algeria.*

Introduction: Chronic inflammatory diseases therapy has been revolutionized since the introduction of three main anti-TNFα drugs (Infliximab, Adalimumab and Etanercept). However, as most genetically engineered proteins, they are immunogenic, inducing the production of anti-drug antibodies (ADA) that lead to drug failure. The aim of our study is to assess the immunogenicity of these 3 anti-TNFα therapies. **Materials and methods:** Our study involved 42 patients (19 females and 23 males, mean age 38±13 years, disease duration 10±6 years) with established Rheumatoid arthritis, Crohn disease or ankylosing spondylitis. All patients presented primary or secondary therapeutic failure after anti-TNFα use. Both drug and ADA levels were measured in patient's sera using an enzyme-linked immuno sorbent assay (ELISA) [Promonitor®Progenika Biopharma SA, Spain]. **Results:** 29% of patients produced ADAs (IFX 36%, ADL 32% and ETN 0%). The presence of ADAs is associated with low drug levels below therapeutic range (>80%). Females (58%) and older patients (60%) are most likely to produce ADAs. Moreover, ADAs develop after a fewer number of injections in IFX patients vs. ADL and in females vs. males. However, the association of Methotrexate (MTX) to the anti-TNFα decreases significantly its immunogenicity. ADA frequency varies with the underlying disease but no significant influence of a previous biotherapy switch has been demonstrated in our study. **Conclusion:** Drug failure to anti-TNF-α therapies is due to ADAs production. This immunogenicity is influenced by numerous factors that are either, drug related (drug structure, association to an immunosuppressant agent) or patient related (gender, age and the underlying disease).

P.C2.07.17

TARGET CELLS OF VEDOLIZUMAB IN PERIPHERAL BLOOD AND GUT MUCOSA CELLS FROM IBD PATIENTS

W. T. C. Uniken Venema, *M. D. Voskuil, A. Bangma, B. H. Jansen, G. Dijkstra, R. K. Weersma, E. A. Festen;
University Medical Center Groningen, Groningen, Netherlands.*

Introduction: The biological vedolizumab (anti-α4β7) blocks the migration of leukocytes into the gut. Treatment results in remission in ~30% of Crohn's disease (CD) and ulcerative colitis (UC) patients. In UC, remission is reached faster than in CD. Deeper insight into the immunological effects of vedolizumab is necessary to explain differences in effect of vedolizumab therapy between CD and UC. Aims and Methods: The aim of this study is to determine the binding capacity of vedolizumab to both immune cells in blood and to isolated mucosal cells from the inflamed gut mucosa. We collected blood and intestinal biopsies from patients with CD and UC prior to vedolizumab treatment, and blood from healthy controls. We engineered fluorescent-labeled vedolizumab and assessed the percentage and level of vedolizumab-binding to cell-subtypes, using flow-cytometry. Results: Vedolizumab binds to a variety of peripheral blood immune cells (*i.e.* $CD4^+$T-cells, $CD8^+$-T cells, B-cells, eosinophils, NK-cells and monocytes). It nearly covers all gut mucosa directed $CD4^+CD38^+CD62L^{neg}$ T cells (median 82% [IQR 68-91]), with significantly higher level of binding than T-cells and B-cells (P<0.0001), and eosinophils (median 91% [IQR 83-94]). No significant differences were observed between patients with CD, UC, and healthy controls. Within the intestinal mucosa, vedolizumab mostly binds lamina propria cells, and in particular $CD8^+$T-cells from the terminal ileum (median 64% [IQR 28-94]). Conclusion: Differences in percentages and level of vedolizumab-binding to cell-subtypes does not explain differences in effect of vedolizumab therapy between CD and UC. These results provide baseline data for correlating vedolizumab binding capacities to clinical response in IBD patients.

P.C2.07.18

IMPORTANCE OF THE RECOGNITION OF THE DENSE FINE SPECKLED PATTERN AND ANTI-DFS70 SPECIFICITY CONFIRMATION IN THE AUTOIMMUNITY LABORATORIES

R. VALENCIA PEREIRA, *A. MARTINEZ RODRIGUEZ, M. ESPARRAGO RODILLA, B. SACRISTAN ENCISO, S. CARRETERO CRUZ, S. GORDILLO VAZQUEZ, M. VARGAS PEREZ;
SERVICIO EXTREMEÑO DE SALUD, BADAJOZ, Spain.*

Introduction: The nuclear dense fine speckled (DFS, AC-2) pattern is associated with autoantibodies directed to DFS-70 antigen. This pattern is recognized as a dense fine speckled staining in the nucleus with a strong staining of mitotic chromosomes, it can be difficult to differentiate from similar patterns such as nuclear homogeneous (AC-1). Therefore, it is crucial to confirm the DFS70 specificity.
The aim of this study was to evaluate the frequency of DFS pattern in our population, its association with the DFS70 antigen and its clinical significance.
Material and methods: The Antinuclear Antibodies (ANA) test by IIF on Hep-2000 cells (Inmunoconcept) was requested in 15759 serum samples from 01/04/2016 to 30/06/2017. From the total number of samples tested, those compatible with DFS70 pattern were selected for further confirmatory testing for anti-DFS70 antibodies (QUANTA Flash DFS70, BIO-FLASH, Inova Diagnostics).
Results: Of the 3674 ANA-positive samples, 118 (3.2%) displayed a DFS pattern. The confirmatory DFS70 test was positive in 67 (56.8%) of the samples tested.
None of the patients DFS70(+) was diagnosed with systemic autoimmune rheumatic disease (SARD), except one patient who was diagnosed with lupus nephropathy, without other positive ANA specificities.
Conclusions: Mono-specific anti-DFS70 antibodies are not associated with SARD.
As the recognition of DFS pattern (AC-2) by IIF is not easy, it is important to confirm the DFS70 specificity with other solid phase immunoassay methods.
Testing for anti-DFS70 antibodies can eliminate unnecessary follow-up testing, being more cost-effective and improving the efficiency of diagnosing SARD.

P.C2.07.19

Blood $CD56^{bright}$ $CD16^+$ NK cells predict calprotectin response to anti-TNFα co-medication with azathioprine in pediatric inflammatory bowel disease

M. Wahlbuhl, *F. Knieling, T. Rechenauer, G. Siebenlist, S. Kaspar, C. Ehrsam, A. Rückelt, W. Rascher, A. Hoerning;
Pediatric Gastroenterology and Hepatology, Erlangen, Germany.*

Introduction: Natural killer (NK) cell subsets have recently been found to play an important role in inflammatory bowel diseases (IBD). We studied NK cell subsets before and after initiation of anti-TNFα therapy and co-medication with azathioprine (AZA) in pediatric IBD.
Materials and Methods: The study was approved by the local ethic committee (ethics protocol #347_15B) and written content was obtained from all guardians. A total of n=21 pediatric IBD patients (Crohn's disease, CD/ulcerative colitis, UC) and n=9 healthy controls (HC) were recruited. Blood samples and intestinal biopsies were collected before initiation of the anti-TNFα therapy and during therapy rather at disease exacerbation. Flow Cytometry (CD3, CD8, CD16, CD56, CD62L, CCR9, b7 integrin), immunofluorescence staining (CD16, CD56), and NanoString (NanoString Technologies, Seattle, WA, USA) technique was performed. The data were compared to the clinical data derived from the medical files.
Results: Before initiation of anti-TNFα therapy in patients already receiving AZA, flow cytometry showed low numbers of peripheral $CD56^{bright}CD16^+$ (CD, p=0.002/UC, p<0.0001), $CD56^{dim}CD16^+$ (CD, p=0.049/UC, p=0.027) and γδT-cells (CD, p=0.42/UC=0.024) in patients with IBD compared to HC. CD-patients reaching a calprotectin <50mg/kg after initiation of anti-TNFα/AZA therapy had a significant higher relative increase of peripheral $CD56^{bright}CD16^+$ cells beginning at 6 (1.42±0.67 vs 0.80±0.39, p=0.0478) and 12 months (1.88±0.81 vs 0.73±0.18, p=0.0005). Double positive $CD56^+CD16^+$ cells were reduced in pediatric IBD intestinal biopsies treated with AZA (CD, p=0.0152/UC, p<0.0001).
Conclusions: Peripheral $CD56^{bright}CD16^+$ NK cell subsets predict calprotectin response in pediatric IBD following anti-TNFα/azathioprine therapy.

P.C2.08 Immune signaling and therapy in autoimmunity - Part 8

P.C2.08.01

Pharmacological activation of pyruvate kinase M 2 inhibits T cell activation and suppresses autoimmunity

S. Angiari, *E. M. Palsson-McDermott, M. C. Runtsch, H. Kane, C. E. Sutton, K. H. Mills, L. A. O'Neill;
Trinity College Dublin, Dublin, Ireland.*

BACKGROUND. Pyruvate kinase (PKM) catalyses the conversion of phosphoenolpyruvate to pyruvate during glycolysis, and previous studies suggested that the PKM isoform PKM2 also has moonlighting activities different from its canonical one. However, its role in $CD4^+$ T cell biology has never been investigated so far. The aim of this study was to study the involvement of PKM2 in T cell activation, effector functions and pathogenic activity. RESULTS. We found that PKM2 is strongly up-regulated in $CD4^+$ T cells following CD3/CD28 activation, can be detected in both the cytoplasm and the nucleus of resting and activated T cells, and is present in equilibrium between a monomeric (inactive) and a tetrameric (active) form. Surprisingly, pharmacological activation of PKM2 with TEPP-46, a small molecule that stabilises the tetrameric form, severely impacted T cell functions, reducing activation, proliferation and cytokine production by activating T cells. This effect was due to inhibition of hypoxia-induced factor-1 α (HIF-1α) and alteration of intracellular metabolism. TEPP-46 also induced the expression of forkhead box p3 (Foxp3) and the generation of regulatory T cells (Tregs) during T cell activation *in vitro*. Importantly, treatment with TEPP-46 blocked the development of experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis, by reducing T cell activation and inducing Treg cells *in vivo*. CONCLUSIONS. Our results suggest that pharmacological activation of the glycolytic enzyme PKM2 may represent a novel valuable toll for the treatment of inflammatory pathologies, and confirm that modulation of immune cell metabolic profile may have therapeutic utility in inflammation.

P.C2.08.02

Regulatory T cells have a distinct migratory phenotype in relapsing-remitting multiple sclerosis patients

***P. Baeten**, N. Hellings, B. Broux;*
Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium.

Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system (CNS). In healthy donors (HD), autoreactive T cells are suppressed by regulatory T cells (Tregs). In relapsing-remitting (RR)MS patients, blood-circulating Tregs are defective. Little is known about their presence in and migration to the CNS. Here, the migratory phenotype and capacity of Tregs are investigated. Using an *in vitro* model of the human blood brain barrier (BBB), we found that Tregs have a migration frequency of 8% across an inflamed BBB. Analysis of paired blood and cerebrospinal fluid (CSF) samples of untreated RRMS patients shows comparable percentages of Tregs (CSF: 6.8%; blood: 8.4%), indicating their capability of migrating to the CNS. To understand the mechanism of migration, Tregs and BBB endothelial cells (EC) were phenotyped. The percentage of $CD11a^+$ Tregs is significantly decreased in blood of untreated RRMS patients (43.3%) compared to HD (51.2%, p=0.0197). In contrast, the CD11a ligand, ICAM-1, is significantly increased on inflamed BBB-EC. With regard to chemokine receptors, we found that the percentage of $CCR5^+$ Tregs is significantly increased in blood of RRMS patients (39.6%) compared to HD (23.7%, p=0.0075). Interestingly, $CCR5^+$, $CXCR3^+$ and $CCR6^+$ Tregs are enriched in the CSF of untreated RRMS patients. The CXCR3 ligand, CXCL10, is significantly upregulated in inflamed EC. These results suggest that Tregs use a specific set of adhesion molecules and chemokine receptors to migrate to the CNS of MS patients. Further results of ongoing analyses will be presented at the meeting.

P.C2.08.03

Infection risk assessment during immunosuppressant treatment using computer simulations

***K. Beuke**, M. Rehberg, A. Dietrich, T. Klabunde, Ö. Sercan Alp, N. Biesemann, C. Asbrand;*
Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany.

Standard of care biologics for inflammatory diseases, such as rheumatoid arthritis, target cytokines and cell activation / development to break the cycle of chronic inflammation and to interfere with disease progression. However, the relief of pain and other symptoms may come at the cost of an increased infection risk. To better understand the increase in infection risk, we developed a model-platform that describes the innate and adaptive immune response to bacterial and viral infections.
On the cellular level, the platform describes activation of macrophages, development of antigen-presenting cells, induction of the cellular and humoral response in draining lymph nodes of the infected tissue, and recruitment of different T cells and B cells to the site of infection, which was calibrated using tissue cell counts. On the molecular level we used a broad range of in vivo and in vitro data for model calibration to capture secretion of key inflammatory cytokines involved in cell activation, differentiation, maturation and recruitment, as well as antibody production.
This ODE-based dynamic immunology platform allows for exploration of treatment regimens and mode of action of standard immunosuppressants, such as TNF-inhibitors (Adalimumab, Etanercept, Infliximab), IL-6-inhibitors (Sirukumab), IL-6R inhibitors (Sarilumab), and B cell depletors (Rituximab), in their role of reducing the immune systems effectiveness in fighting infections such as dormant tuberculosis.
For Sanofi, the platform is an holistic approach to infer mechanistic understanding from sparse data with a certain predictive power for drug development with orthogonality to current animal models.

P.C2.08.04

GLUTATHIONE CONTROLS ROS TO PRIME T CELLS FOR INFLAMMATION

*T. W. Mak[1], M. Grusdat[2], G. Duncan[1], C. Dostert[2], C. Binsfeld[2], M. Cox[1], C. Gorrini[1], Z. Hao[1], M. Itsumi[3], Y. Nonnenmacher[4], C. Jäger[5], A. Brüstle[6], M. Ollert[2], Y. Chen[7], B. Camara[8], C. Bindslev-Jensen[9], P. Lang[10], O. Pinkenburg[8], V. Vasiliou[7], M. Lohoff[8], I. Harris[11], K. Hiller[4], **D. Brenner**[2];*
[1]The Campbell Family Institute for Breast Cancer Research, Toronto, Canada, [2]LIH, Esch-sur-Alzette, Luxembourg, [3]Tokyo Medical and Dental University, Tokyo, Japan, [4]University of Braunschweig, Braunschweig, Germany, [5]University of Luxembourg, Esch-sur-Alzette, Luxembourg, [6]Australian National University, Canberra, Australia, [7]Yale School of Public Health, New Haven, United States, [8]University of Marburg, Marburg, Germany, [9]Odense University Hospital, Odense, Denmark, [10]University of Düsseldorf, Düsseldorf, Germany, [11]Harvard Medical School, Boston, United States.

Activated T cells produce reactive oxygen species (ROS), which trigger the antioxidative glutathione (GSH) response necessary to buffer rising ROS and prevent cellular damage. We report that GSH is essential for T cell effector functions through its regulation of metabolic activity. Conditional gene targeting of the catalytic subunit of glutamate cysteine ligase (Gclc) blocked GSH production specifically in murine T cells. Gclc-deficient T cells initially underwent normal activation but could not meet their increased energy and biosynthetic requirements. GSH deficiency compromised the activation of mammalian target of rapamycin-1 (mTOR) and expression of NFAT and Myc transcription factors, abrogating the energy utilization and Myc-dependent metabolic reprogramming that allows activated T cells to switch to glycolysis and glutaminolysis. In vivo, T-cell-specific ablation of murine Gclc prevented autoimmune disease but blocked antiviral defense. The antioxidative GSH pathway thus plays an unexpected role in metabolic integration and reprogramming during inflammatory T cell responses.

P.C2.08.05

The diagnostic value of the antiaging protein, Klotho in early and late stages of multiple sclerosis

***M. Emami Aleagha**[1], A. Allameh[1], M. Harirchian[2], S. Rostami[1], S. Lavasani[3], M. Javan[4], M. Pahlevan Kakhki[5];*
[1]Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, Islamic Republic of, [2]Iranian Center of Neurological Research, Neuroscience Institute, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of, [3]Department of Biology, Sölvegatan 35, Building C, SE-223 62, Lund, Sweden, [4]Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, Islamic Republic of, [5]Department of Clinical Neuroscience, Center for Molecular Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.

Introduction: Expression of an anti-aging protein, i.e Klotho in the CNS, is influenced by inflammatory cytokines such as TNF-α and IFN-γ. On the other hand, in inflammatory diseases Klotho exerts its immunomodulatory effects such as the suppression of TNF-α-induced expression of adhesion molecules and NF-κB activation. The aim of the present study was to investigate the relationship of Klotho expression at protein and mRNA levels with the pathogenesis of MS with emphasize on its diagnostic value.
Methods: Klotho protein level was estimated in cerebrospinal fluid (CSF) from patients with relapsing-remitting MS (RRMS). Changes in klotho was also verified in the brain and spinal cord during distinct stages of experimental autoimuune encephalomyelitis (EAE) development in mice.
Results: The results of the clinical study showed that CSF level of klotho is inversely related to the MS severity in RRMS patients. Accordingly, *Klotho* gene expression in peripheral blood mononuclear cells (PBMCs) was significantly lower in RRMS patients as compared to healthy individuals. The data from EAE mice revealed that *Klotho* gene expression in the brain was dramatically decreased, while there was a significant increase in the expression of *Klotho* gene in spinal cord tissue.
Conclusion: Perhaps, the brain and spinal cord in case of EAE mice exhibited different sensitivities to inflammatory cytokines such as, IFN-γ and IL-17. Hence, it seems that Klotho responds differently to the inflammatory pathways during the MS and EAE pathogenesis. These results may suggest that the Klotho is a potent candidate for diagnosis, monitoring and also treatment of MS.

P.C2.08.06

IMMUNOMODULATORY PROPERTIES OF STROMAL MESENCHYMAL STEM CELLS DERIVED FROM HUMAN AND MURINE FALLOPIAN TUBE IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)

***C. L. Freitas**[1], C. M. Polonio[1], W. N. Brandão[1], N. G. Zanluqui[1], L. G. Oliveira[1], C. Czeresnia[2], L. P. Evangelista[2], S. Halpern[2], M. G. Nisenbaum[2], M. Maluf[3], P. Perin[3], J. S. Peron[1];*
[1]Institute of Biomedical Sciense, São Paulo, Brazil, [2]Célula Mater, São Paulo, Brazil, [3]Clínica Especializada em Reprodução Humana – CEERH, São Paulo, Brazil.

INTRODUCTION: Multiple sclerosis (MS) is a neurodegenerative autoimmune disease that leads to demyelination of neuronal axons. Fallopian tubes are a source of mesenchymal stromal cells in humans (htMSCs) and mice (meMSCs), wich are undifferentiated multipotent cells that play an important role in autoimmune diseases due its immunomodulatory properties. Since the management of symptoms remains unclear, we propose to evaluate the immunomodulatory effect of htMSCs and meMSCs using the murine model of MS, the Experimental Autoimmune Encephalomyelitis (EAE). **METHODS**: C57BL/6 mice were immunized with MOG_{35-33} and treated or not with htMSCs and meMSCs. At different post-immunization times mice were euthanized, CNS-infiltrating cells were analysed by flow citometry; spinal cords were analyzed by PCR and histology. **RESULTS**: Our data show a significant reduction in EAE scores of htMSC/meMSC treated mice, demonstrating its modulatory effect *in vivo*. We observed fewer infiltrating cells in the CNS in the htMSC/meMSC-treated mice, correlated with reduced number of macrophages, Th1 and Th17 cells. This group also presented less activated microglia with lower levels of MHC II and CD80 expression. The spinal cords showed reduced expression of Ifng and Il17 genes, less infiltrating cells and demyelination compared to control group. Furthermore, analysis *in vitro* showed that the meMSC are able to decrease of proinflammatory cytokines production by $TCD4^+$ cells. **CONCLUSION:** Altogether, our data shows that htMSCs treatment is able reduce neuroinflammation in EAE model. The modulatory effect of *htMSC* and meMSC cells are promising and can lead to a future strategy of treatment of autoimmunity or neurodegenerative diseases.

P.C2.08.07

Combinatorial associative study of IL10, IL18 and TNF-α gene polymorphisms in relapsing-remitting multiple sclerosis

B. Grigorov[1], *A. Trenova*[2], *L. Miteva*[1], *S. Stanilova*[1];
[1]*Medical Faculty, Trakia University, Stara Zagora, Bulgaria,* [2]*Faculty of Medicine, Medical University, Plovdiv, Bulgaria.*

Introduction: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system leading to neurological dysfunctions. The aim of the present study was to investigate the association between the promoter polymorphisms in IL10 (rs1800896), IL18 (rs1946518), TNF-α (rs1800629) and genetic predisposition to relapsing-remitting multiple sclerosis (RRMS) in Bulgarian patients.
Materials and Methods: Our case-control study includes 159 RRMS patients with DMT therapy (114 women and 45 men, 40.08±8.48 years) and 125 women and 44 men (39.27±9.85 years) age-sex-matched healthy volunteers. All included subjects were genotyped by ARMS-PCR and RFLP-PCR methods.
Results: Our results revealed no significant differences of studied polymorphisms between cases and controls: IL10 (χ^2=0.493; p=0.782); IL18 (χ^2 =4.073; p=0.130) and TNF-α (χ^2= 3.153; p=0.207). However, we must note that homozygous for variant allele-A in IL18 polymorphism was not detected in the patient's group in contrast to control. Also, for rs1800629 in TNF-α, it was calculated OR=1,505 for the heterozygous genotype. When we combined both IL10 and IL18 polymorphisms, we detected the lower frequency of AG+GG/CC genotypes compared to the wild genotypes AA/CC (OR=0.481, 95% CI=0.199÷1.157, p=0.073). In the same line were the results when was added the wild genotype of TNF-α polymorphism (GG) to the combination of IL18 and IL10 polymorphisms (GG/AA/CC vs GG/AG+GG/CC, OR=0.446, 95% CI=0.165÷1.189, p=0.073).
Conclusion: Our present results indicate that the carrying of variant allele-G in IL10 (AG+GG genotypes) in combination whit both wild genotypes of IL18, and of TNF-a polymorphisms, might influence the risk of RRMS susceptibility in Bulgarian patients.

P.C2.08.08

Association study of HLA class I antigens with central nervous system inflammatory diseases

S. Mejdoub[1], *A. Charfi*[2], *A. Kamoun*[2], *N. Mahfoudh*[2], *S. Feki*[1], *M. Dammak*[3], ***H. Hachicha***[1], *L. Maalej*[2], *I. Kamoun*[2], *F. Hakim*[2], *L. Gaddour*[2], *B. Mallek*[2], *C. Mhiri*[3], *H. Masmoudi*[1], *H. Makni*[2];
[1]*Immunology Laboratory, Habib Bourguiba Hospital, Sfax, Tunisia,* [2]*Histocompatibility Laboratory, Hedi Chaker Hospital, Sfax, Tunisia,* [3]*Neurology Department, Habib Bourguiba Hospital, Sfax, Tunisia.*

Central nervous system (CNS) inflammatory diseases include multiple sclerosis (MS) but also neurological manifestations of systemic diseases such as Behçet disease. As the association of HLA-B51 antigen with Behçet disease is well established, our aim was to evaluate the frequency of this antigen in patients with CNS inflammatory disease suspicion. Patients followed in neurology department for CNS inflammatory disease suspicion (June 2014-May 2016) for whom HLA class I typing was performed (microlymphocytotoxicity complement dependent technique) were included. Our control population included 123 unrelated healthy subjects. Statistics were studied according to Microsoft Excel.
Forty patients (17 men and 23 women) were included. The established diagnosis was MS in 27 cases, clinically isolated syndromes (CIS) in 8 cases, Behçet disease in 2 cases and CNS vascular disease in 3 cases. Among these patients, 10 (7 MS and 3 CIS) expressed HLA-B51 antigen (25% VS 9,76% in controls, *p*=0,01; OR=3,08). Comparing MS patients with controls, HLA-A10 antigen was significantly associated with the disease (25,93% VS 9,76%, *p*=0,04; OR=3,24). All patients expressing HLA-A10 antigen belonged to MS group.
Our study, despite concerning a small number, showed an association of HLA-B51 antigen with CNS inflammatory diseases particularly MS. This can probably be explained by linkage disequilibrium between this antigen and HLA-DR15, reported as a susceptibility marker for MS. Association of HLA-A10 with this disease suggests probably the involvement of another susceptibility region telomeric to HLA-B locus. A bigger patients population size is required to confirm our preliminary results.

P.C2.08.09

Regulatory T cell-driven suppression of experimental autoimmune encephalomyelitis during pregnancy

N. Heckmann[1], *J. B. Engler*[1], *C. Ramien*[1], *S. M. Gold*[1,2], *M. A. Friese*[1];
[1]*Institut für Neuroimmunologie und Multiple Sklerose, Zentrum für Molekulare Neurobiologie Hamburg, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany,* [2]*Charité Universitätsmedizin, Berlin, Germany.*

Pregnancy reduces disease activity of several inflammatory autoimmune diseases including multiple sclerosis (MS), where the relapse rate is reduced by approx. 80% in the third trimester. This protection is mimicked in experimental autoimmune encephalomyelitis (EAE), the animal model of MS. Regulatory T cells (Treg) play a pivotal role in controlling both feto-reactive T cells during pregnancy and auto-reactive T cells in autoimmune diseases, however, it remains unknown how Treg exert their increased suppressive capacity during pregnancy. We have recently reported that Treg increase during pregnancy and that the protective effect on EAE is dependent on regulation via the glucocorticoid receptor in T cells. In the present study, we surveyed energy metabolism, phenotype and T cell receptor (TCR) repertoire of Treg during pregnancy to determine the relative contributions of these factors to pregnancy-induced Treg activity and protection from autoimmunity. We detected no changes in energy metabolism in Treg during pregnancy in mice. Next, we probed the TCR repertoire of Treg and Tcon at different pregnancy stages and under EAE conditions and are currently monitoring the relative distribution of suppressive clones. To understand Treg suppressive capacity and differentiation changes during pregnancy, we extensively phenotyped and functionally analysed Treg from pregnant and non-pregnant EAE mice. Together, our findings give a comprehensive understanding of the pregnancy-induced changes in Treg and how these changes contribute to an amelioration of EAE disease activity.
This work is funded by the DFG KFO 296.

P.C2.08.10

Multiple Sclerosis associated cytotoxic CD4$^+$T cells escape regulatory T cell mediated suppression

C. Hoeks*, M. Vanheusden, L. Peeters, P. Stinissen, B. Broux, N. Hellings;*
Hasselt University, Biomedical Research Institute and Transnationale Universiteit Limburg, Diepenbeek, Belgium.

A terminally differentiated subset of CD4$^+$ T lymphocytes, characterized by loss of the costimulatory molecule CD28 and gain of cytotoxic activity, arises during aging and chronic inflammation. An age-inappropriate expansion of these cells has been found in autoimmune diseases like multiple sclerosis (MS). Our group has recently published that CD4$^+$ cytotoxic T lymphocytes (CTL) contribute to the pathology of MS. We showed that expansion of CD4+ CTL exacerbates experimental autoimmune encephalomyelitis. In addition, we found that presence of peripheral CD4+ CTL is directly linked to MS disease severity and that this holds value as a novel prognostic marker in MS. However, the mechanism behind these findings remains unclear. Here we show that CD4$^+$CD28^{null} T cells are phenotypically distinct from CD4$^+$CD28$^+$T cells, and that CD4$^+$CD28^{null} T cells evade Treg-mediated suppression *in vitro.* CD4$^+$CD28^{null} T cells display enhanced levels of pro-inflammatory molecules such as granzyme B, IFN-gamma, IL-1beta, IL-6, IL-22, and GM-CSF, but decreased levels of IL-10R and GITR. Tregs upregulate IL-10, granzyme B, CTLA-4, and IFN-gamma when exposed to the secretome of CD4$^+$CD28^{null} T cells. An *in vitro* co-culture system has been optimized to further analyze how CD4$^+$CD28^{null} T cells affect Tregs and vice versa. Our results suggest that CD4$^+$CD28^{null} T cells can evade Treg suppression through two distinct mechanisms: 1) by becoming less susceptible to Treg activity and 2) by directly altering the functionality of Tregs. Elucidating these pathways may contribute to the development of novel therapeutic interventions specifically targeting age-inappropriate expansion of CD4$^+$ CTL in autoimmune diseases like MS.

P.C2.08.11

B cell subpopulations in the pathogenesis of multiple sclerosis

R. Holm Hansen*, O. Mcwilliam, H. Højsgaard Chow, F. Sellebjerg, M. R. von Essen;*
Danish Multiple Sclerosis Center, Copenhagen Ø, Denmark.

B cells are important contributors to the pathogenesis of multiple sclerosis (MS), where they regulate the inflammatory immune response and participate in development of lesions in the CNS. Dimethyl fumarate (DMF) is used to treat patients with relapsing-remitting MS (RRMS); however, its impact on B cell subpopulations remains uncertain. In this study we therefore investigated the phenotype of B cell subpopulations in 18 untreated patients with RRMS, 17 healthy controls (HC) and 21 patients treated with DMF for more than 12 months. Using flow cytometry, B cell subpopulations were defined according to their expression of CD27 and CD38. This showed that CD27-CD38- B cells were increased in the blood of untreated patients compared to HC. We also found that CD27-CD38- B cells migrated to the CSF of untreated patients; proposing an association with MS pathogenesis. When patients were treated with DMF we found a treatment-induced reduction in the frequency of CD27-CD38- B cells and CD27+ memory B cells. We also found that the observed reduction in antigen-experienced B cells in DMF-treated patients likely was due to a reduced frequency of follicular helper T (T_{FH}) cells and an increased frequency of follicular regulatory T cells, whose function is to restrain the activity of T_{FH} cells. Studying the impact of fumarate on B cell cytokine production showed that it decreased the frequency of LTα, TNFα, IL-6 and IL-10 producing B cells and increased the frequency of TGF-β producing cells. These data demonstrate an anti-inflammatory role of DMF on the B cell compartment.

P.C2.08.12

Targeting Plasma Cells with Proteasome Inhibitors for Treatment of Myasthenia Gravis

*M. Mané Damas, **M. Losen**, M. Pilar;*
Maastricht University, Maastricht, Netherlands.

Autoantibodies against the muscle AChR are mainly produced by both short- and long-lived plasma cells, which are resistant to standard immunosuppressive drugs (*e.g.* glucocorticoids). A novel therapy to eliminate plasma cells is the proteasome inhibitor bortezomib, which is used to treat patients with multiple myeloma (MM, a plasma cell malignancy). Previously, we demonstrated that bortezomib also reduced autoantibody titers in an animal model of MG (Gomez, A. M. *J. Immunol*. 2011). The thymus of MG patients is frequently enriched in germinal centers and contains plasma cells that produce autoantibodies *in vitro*, even after irradiation (which depletes B and T lymphocytes). We studied the *in vitro* effects of bortezomib in cultured thymus cells from MG patients undergoing therapeutic thymectomy. Treatment with a single dose of bortezomib eliminated plasma cells and thereby blocked the production of IgG, including pathogenic autoantibodies. Ultrastructural signs of apoptosis were detected in plasma cells as early as 8 h after addition of bortezomib; at 24 h, no plasma cells could be detected (Gomez, A. M. *J.Immunol*. 2014). Finally, we are currently testing in vitro and in vivo second-generation proteasome inhibitors efficient in eliminating autoreactive plasma cells with special focus in investigating their side effects such as peripheral neuropathy.

P.C2.08.13

Anti-NF155 chronic inflammatory demyelinating polyradiculoneuropathy strongly associates to HLA-DRB15

***L. Martinez-Martinez**[1], M. Lleixà[2], G. Boera-Carnicero[1], A. Cortese[3], J. Devaux[4], A. Siles[5], Y. Rajabally[6], A. Martinez-Piñeiro[7], A. Carvajal[8], J. Pardo[9], J. Diaz-Manera[5], I. Callegari[3], E. Marchioni[3], D. Franciotta[3], L. Benedetti[10], G. Lauria[11], C. Juárez[1], I. Illa[5], L. Querol[5], Ó. de la Calle-Martin[1];*
[1]Immunology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, [2]Neuromuscular Diseases Unit, Neurology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, [3]IRCSS Foundation C. Mondino National Neurological Institute, Pavia, Italy, [4]Centre de Recherche en Neurobiologie et Neurophysiologie, Marseille, France, [5]Neuromuscular Diseases Unit , Neurology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, [6]Regional Neuromuscular Clinic, Queen Elizabeth Hospital, Birmingham, United Kingdom, [7]Neurology, Hospital Germans Trias i Pujol, Badalona, Spain, [8]Neurology, Hospital Virgen de las Nieves, Granada, Spain, [9]Neurology, Hospital Clínico de Santiago, Santiago de Compostela, Spain, [10]Neuroscience, University of Genova and IRCCS AOU San Martino-IST, Genova, Italy, [11]Neuroalgology Unit, IRCCS Foundation Carlo Besta Neurological Institute, Milan, Italy.

OBJECTIVE: To study the human leukocyte antigen (HLA) class II allele frequencies in chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) associated with anti-neurofascin 155 (NF155) antibodies. METHODS: Thirteen anti-NF155+ and 35 seronegative CIDP patients were included in a case-control study. The frequencies of the DRB1 HLA allele were analyzed in all patients while DQ frequencies were only studied in patients sharing the DRB1*15 allele. In silico HLA-peptide binding and NF155 antigenicity predictions were performed to analyze overlap between presented peptides and antigenic regions. RESULTS: DRB1*15 alleles (DRB1*15:01 and DRB1*15:02) were present in 10 (out of 13) anti-NF155+CIDP patients and in only 5 (out of 35) seronegative CIDP patients (76.9% vs 14.2%; OR=20, CI=4.035 to 99.13). DRB1*15 alleles appeared also in significantly higher proportions in anti-NF155+CIDP than in normal population (76.9% vs 16.5%; OR=16.9, CI= 4.434 to 57.30). Seven anti-NF155+ CIDP patients (53%) and 5 seronegative CIDP patients had the DRB1*15:01 allele (OR=7, p=0.009), while 3 anti-NF155+ CIDP patients and none of the seronegative CIDP patients had the DRB1*15:02 allele (OR=23.6, p=0.016). In silico analysis of the NF155 peptides binding to DRB1*15 alleles showed significant overlap in the peptides presented by the 15:01 and 15:02 alleles, suggesting functional homology. CONCLUSION: DRB1*15 alleles associate strongly to anti-NF155 antibodies in CIDP and provide additional evidence to support that these patients constitute a differentiated CIDP subset.

P.C2.08.14

Teriflunomide induces in multiple sclerosis a tolerogenic phenotype in innate immune cells resulting in a reduction in terminally differentiated effector lymphocytes.

***S. Medina**[1], N. Villarrubia[1], S. Sainz de la Maza[1], R. Alvarez-Lafuente[2], A. Tejeda-Velarde[1], L. Costa-Frossard[1], E. Rodriguez-Martin[1], R. Arroyo[3], J. C. Álvarez-Cermeño[1], E. Roldán[1], L. Villar[1];*
[1]Ramón y Cajal Hospital, Madrid, Spain, [2]Clínico San Carlos Hospital, Madrid, Spain, [3]Quirónsalud Madrid Hospital, Madrid, Spain.

Introduction: Teriflunomide is a disease modifying treatment approved for multiple sclerosis (MS). This molecule inhibits reversibly dihydro-orotate dehydrogenase, a mitochondrial enzyme involved in de-novo pyrimidine biosynthesis, and down-regulates proliferation of activated lymphocytes. However, there are few data on the impact of this drug on the lymphocyte profiles of MS patients. We further studied this in a cohort of MS patients treated with teriflunomide.
Methods: 55 patients with relapsing-remitting MS who initiated teriflunomide treatment were included in the study. We studied peripheral blood mononuclear cells obtained before and six months after treatment initiation and explored effector, memory and regulatory cells by flow cytometry.
Results: When explored T and B cell subsets, we observed a decrease in the percentages of terminally differentiated CD4+ T cells (p=0.001) and plasmablasts (p<0.0001) after 6 months of treatment. These results were confirmed with the total cell number. When studied regulatory cells, we observed a clear increase of monocytes expressing programmed death-ligand 1 (PDL-1) (p=0.005), which correlated negatively with all effector CD8+ T cell subsets. We also observed an increase in the percentage of CD8+ T cells (p=0.028) and monocytes (p=0.04) producing IL-10.
Conclusions: Teriflunomide induces a specific reduction in effector T and B cells that have shown to play a role in MS course and an increase in regulatory cells. This drug induces the expression of PD-L1, a molecule involved in tolerance to autoantigens, which can contribute to inhibit the abnormal immune response taking place in MS.

P.C2.08.15

Biomarkers for early prediction of dimethyl fumarate associated lymphopenia in multiple sclerosis

***S. Medina**, S. Sainz de la Maza, N. Villarrubia, E. Rodríguez- Martín, L. Costa-Frossard, A. Tejeda Velarde, E. Monreal, J. C. Álvarez-Cermeño, E. Roldán, L. Villar;*
Ramón y Cajal Hospital, Madrid, Spain.

Introduction: Dimethyl fumarate (DMF) is a first line treatment for relapsing remitting multiple sclerosis (MS). Lymphopenia is a major concern in MS patients treated with DMF as increases the risk of serious infectious side effects. Predicting which patients have an increased risk of developing lymphopenia could have important implications for personalized therapy in these patients. The main goal was to identify factors predicting lymphopenia in DMF-treated patients.
Methods: Prospective longitudinal study including 106 patients initiating DMF treatment. They were followed for a mean time of 23.58 months, and monitored every three months. Blood lymphocyte subsets were studied in a representative group of 64 patients by flow cytometry at baseline and 6 months after.
Results: Mean absolute lymphocyte counts (ALCs) decreased by 29% during the first year of DMF-treatment. Patients developing lymphopenia showed a faster decline within the three first months. A reduction of ALCs higher than 36% at this time, accurately predicted subsequent lymphopenia (OR=7.35, 95% CI: 3.0-17.9, p<0.0001).
We classified patients in two groups according to the appearance of lymphopenia. Both showed a significant decrease in effector memory T cells, total and terminally differentiated CD8+ T cells and memory B cells upon DMF therapy. In addition, non-lymphopenic patients experienced a selective increase in naïve CD4+ T cells, not experienced by those developing lymphopenia.
Conclusions: A decline in ALCs below 36% after 3 months of DMF-treatment identifies patients with low probability of developing lymphopenia. This decrease may be associated with retardation in the production of naïve CD4+ T cells.

P.C2.08.16

The inhibition of dopamine receptor D3 signalling in CD4$^+$ T-cells exerts a therapeutic effect attenuating Parkinson's disease development in a mouse model

***R. Pacheco**[1,2], D. Elgueta[1,2], F. Contreras[1], C. Prado[1], A. Montoya[1], M. A. Abellanas[3], M. S. Aymerich[3], R. Franco[4];*
[1]Fundación Ciencia & Vida, Santiago, Chile, [2]Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas, Universidad Andres Bello, Santiago, Chile, [3]Division of Neurosciences, Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain, [4]Department of Biochemistry and Molecular Biomedicine, University of Barcelona, Barcelona, Spain.

Neuroinflammation constitutes a fundamental process involved in Parkinson's disease (PD). Microglial cells play a central role in the outcome of neuroinflammation and consequent neurodegeneration of dopaminergic neurons in the substantia nigra. Current evidence indicates that CD4$^+$ T-cells infiltrate the brain in PD, where they play a critical role determining the functional phenotype of microglia, thus regulating the progression of the disease. Recently, we demonstrated that mice bearing dopamine receptor D3 (DRD3)-deficient CD4$^+$ T-cells are completely refractory to neuroinflammation and consequent neurodegeneration associated to the intoxication with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In this study we evaluated the therapeutic potential of targeting DRD3 in CD4$^+$ T-cells by inducing the pharmacologic or the transcriptional inhibition of DRD3-signalling in a mouse model of PD induced by the chronic administration of MPTP and probenecid (MPTPp). We also analysed whether DRD3-signalling was altered in the immune cells of PD patients. The results show that the transference of CD4$^+$ T-cells transduced *ex vivo* with retroviral particles codifying for an shRNA for DRD3 or treated *ex vivo* with the DRD3-selective antagonist PG01037 into MPTPp-mice resulted in a significant reduction of motor impairment and neuroinflammation. *In vitro* analyses showed that the frequency of peripheral blood Th1 cells, a phenotype that is promoted by DRD3-signalling, was significantly increased in PD patients. Nevertheless, DRD3 expression was selectively reduced in CD4$^+$ T-cells obtained from PD patients. Our findings indicate that attenuating DRD3-signalling in CD4$^+$ T-cells exerts a therapeutic effect in parkinsonian animals dampening motor impairments and modifying the pro-inflammatory phenotype of glial cells.

P.C2.08.17

A fusion protein of IL4 and IL10 to resolve inflammatory pain

J. Prado[1], *R. H. Westerink*[2], *J. Popov-Celeketic*[1], *C. Steen-Louws*[1], *A. Pandit*[1], *W. Worp van de*[1], *K. A. Reedquist*[1], *L. Koenderman*[1], *C. E. Hack*[1], *N. Eijkelkamp*[1];
[1]*UMC Utrecht, Utrecht, Netherlands,* [2]*Institute for Risk Assessment Sciences, Utrecht, Netherlands.*

Chronic pain is difficult to treat and new therapeutic approaches to treat it are highly needed. Anti-inflammatory cytokines have potential to resolve chronic pain, but they work most optimal in concert of each other. We developed a novel approach to optimize the potential of anti-inflammatory cytokines to resolve persistent pain by fusing IL4 and IL10 into one molecule. Intrathecal administration of IL4-10 fusion protein (FP) completely resolves inflammatory pain in multiple animal models, in a superior fashion than the combination of individual cytokines. In addition, *in vitro*, IL4-10 FP inhibited TNFα and PGE_2-induced neuronal sensitization of capsaicin-induced calcium fluxes. Importantly, equimolar concentrations of IL4-10 FP more effectively inhibited neuronal sensitization than the combination of individual cytokines. Mechanistically, we show that IL4-10 FP, in contrast to the combination of IL4 and IL10, clusters IL4R and IL10R in sensory neurons as a potential mechanism for the increased effectiveness. *In vivo*, conditional knockdown of IL10R in Nav1.8+ nociceptors and IL4R in sensory neurons using intrathecal antisense oligodeoxynucleotides completely ablated the IL4-10-mediated inhibition of inflammatory pain. Knockdown of IL4R or IL10R only was sufficient to prevent the superior effect of IL4-10-mediated pain inhibition. Finally, intrathecal administration of IL4-10 FP induced a completely different kinome and transcriptome profile in the DRG compared to IL4+IL10 treatment. These data underscore that anti-inflammatory cytokines can be used to target the sensory system to fully resolve persistent pain. Moreover, they show that IL4-10 FP achieved unheralded biological effects that are more than the mere sum of the two cytokines.

P.C2.08.19

Assessment of anti-DFS70 antibodies in the diagnostic approach of Systemic Autoimmune Rheumatic Diseases

A. Tsirogianni[1], *A. Giannakou*[2], *K. Soufleros*[1], *M. Bantadaki*[2], *E. Synodinou*[1], *A. Markantonatou*[2], *E. Pipi*[1], *A. Pavlitou*[2];
[1]*Immunology-Histocompatibility Dept. "Evaggelismos" General Hospital, Athens, Greece,* [2]*Immunology-Histocompatibility Dept. "Papageorgiou" General Hospital, Thessaloniki, Greece.*

Antinuclear antibodies (ANA) are a serological hallmark of systemic autoimmune rheumatic diseases (SARD). However, positive ANA with a dense fine speckled (DFS) pattern (Hep-2), have been reported in other clinical entities. Interestingly, the anti-DFS70 antibodies are not significantly associated with SARD.
Aim: The aim of the study was the investigation whether the use of a protocol that pre-absorbs anti-DFS70 antibodies from the sera, increases the diagnostic efficiency of HEp-2 ANA testing for SARD.
Subjects and methods
Positive ANA-IIF consecutive sera with DFS pattern from 120 patients with SARD and 170 patients with other clinical entities (non-SARD) referred to our Departments for ANA, DNA and extractable nuclear antigen (ENA) antibodies testing, were retrospectively tested using the NOVA Lite® HEp-2 Select* kit for ANA-IIF.
Results: The positive ANA-IIF after anti-DFS70 pre-absorption was 94.1% (113/120) in SARD patients, while in non-SARD patients the positivity dropped to 44.7% (79/170). Among the 7 SARD patients who became negative after anti-DFS70 pre-absorption, 3 were diagnosed with cutaneous lupus (dsDNA and ENA negative), 1 with myositis (SSA and Jo-1 positive), 2 with Raynaud syndrome (ENA negative) and 1 with Rheumatoid Arthritis (anti-CCP positive).Among non-SARD patients, about half of those (76/170) who remained ANA positive (DNA and ENA negative), were diagnosed with an autoimmune/inflammatory disease (e.g. inflammatory bowel disease, autoimmune hepatitis, multiple sclerosis).
Conclusions: Based on our results we recommend that suggest that the anti-DFS70 pre-absorption protocol on ANA-IIF increases ANA-Hep-2 specificity for SARD, but more studies are needed to address whether it decreases the respective sensitivity.

P.C2.08.20

Interaction between the HLA-Shared Epitope (SE) and smoking in anti-CCP positive Greek patients with Rheumatoid Arthritis

K. Tarassi[1], *E. Mole*[2], *V. Kitsiou*[1], *D. Kouniaki*[1], *T. Athanassiades*[1], *K. Soufleros*[1], *E. Synodinou*[1], *A. Petsas*[1], *C. Sfontouris*[2], ***A. Tsirogianni***[1];
[1]*Immunology-Histocompatibility Dept. "Evaggelismos" General Hospital, Athens, Greece,* [2]*Rheumatology Dept. "Evaggelismos" General Hospital, Athens, Greece.*

Genetic and environmental factors involve in etiopathogenesis of Rheumatoid Arthritis(RA). The aim of the study was the assessment of association of HLA-DRB1*-SE in the presence/absence of CCP autoimmunity in Greek patients with RA (smokers and non-smokers). Eighty-three (83) RA patients (41 smokers,42 have never smoked) were typed for HLA-DRB1* alleles by molecular techniques (PCR-SSOP and -SSP). In 62 out of 83 (74.7%) anti-CCP abs were detected by ELISA. In RA patients and in comparison to the controls increased frequency of HLA-DRB1*01:01(28.9%vs6.8%,OR=4.4), *10:01(16.9%vs2.4%,OR=8.4), *04:01(3.6%vs2%,OR=1.8), *04:04(7.2%vs1%,OR=7.6) and *04:05(15.7%vs3.7%,OR=4.8), as well as decreased frequency of *04:02(1.2%vs2%,OR=0.6) and *04:03 (4.8%vs6.8%,OR=0.7) were found. Among RA patients, 77.1 % possess 1SE vs 18.9% in controls (OR=14.4), whereas 10.8 % possess 2SE vs 1% in controls (OR=11.8). In CCP(+) RA patients and in comparison to CCP(-), an increased frequency of HLA-DRB1*01:01(27.4%vs14.3%,OR=2.3) and *10:01(21%vs4.8%,OR=5.3) was observed. Furthermore, 88.7% of CCP(+) carry 1SE vs 42.9% of CCP(-), (OR=10.5). CCP(+) smokers patients and in comparison to CCP(+) non-smokers are presented with an increased frequency of DRB1*01:01 (41.9%vs12.9%,OR=4.9). Among the CCP(+) smokers, 96.8% possess 1SE vs 80.6% of CCP(+) non-smokers (OR=7.2), whereas 12.9% possess 2SE vs 12.9% of CCP(+) non-smokers (OR=1). Conclusions: a) An increased frequency of HLA-DRB1*01:01, *10:01, *04:05 alleles, as well as the protective role of *04:02, *04:03 alleles, in Greek patients with RA were confirmed, b) Presence of any SE, particularly *10:01 allele, strongly influences the production of anti-CCP abs, c) Interaction between smoking and any SE, particularly *01:01 allele, is associated with anti-CCP(+) RA in Greek patients.

P.C2.08.21

Dithiolethione ACDT suppresses neuroinflammation and ameliorates disease severity in experimental autoimmune encephalomyelitis

P. Kuo[1], *D. A. Brown*[2], *B. A. Scofield*[1], *H. C. Paraiso*[1], *I. Yu*[1], ***J. Yen***[1];
[1]*Indiana University School of Medicine, Fort Wayne, United States,* [2]*Manchester University College of Pharmacy, Fort Wayne, United States.*

Introduction: Multiple sclerosis (MS) is an autoimmune disorder characterized by the central nervous system (CNS) infiltration of myelin-specific pathogenic T cells followed by brain inflammation in association with demyelination. Similarly, experimental autoimmune encephalomyelitis (EAE), the animal model of MS, also exhibits increased CNS infiltration of pathogenic T cells, including Th1 and Th17, leading to detrimental effects of neuroinflammation and demyelination. We previously reported that 3H-1,2-dithiole-3-thione (D3T), the structurally-simplest of the sulfur-containing dithiolethiones, exerted a promising therapeutic effect in EAE. In the current study we evaluated the therapeutic effect of 5-Amino-3-thioxo-3H-(1,2)dithiole-4-carboxylic acid ethyl ester (ACDT), a substituted derivative of D3T, in EAE.
Materials and Methods: Chronic C57BL/6 EAE and relapsing-remitting SJL/J EAE were induced and treated with ACDT. The effect of ACDT on pathogenic T cell infiltration, microglia activation, neurotoxic A1 astrocytes, blood-brain barrier (BBB) integrity in the CNS of EAE was assessed.
Results: ACDT, administered post immunization, delayed disease onset and reduced disease severity in chronic C57BL/6 EAE, and ACDT, administered during disease remission, suppressed disease relapse in relapsing-remitting SJL/J EAE. Further analysis of the cellular and molecular mechanisms underlying the protective effects of ACDT in EAE revealed that ACDT inhibited pathogenic T cell infiltration, suppressed microglia activation, repressed neurotoxic A1 astrocyte generation, lessened BBB disruption, and diminished MMP3/9 production in the CNS of EAE.
Conclusions: We demonstrate that ACDT suppresses neuroinflammation and ameliorates disease severity in EAE through multiple cellular mechanisms. Our findings suggest the potential of developing ACDT as a novel therapeutic agent for the treatment of MS/EAE.

P.C2.09 Immune signaling and therapy in autoimmunity - Part 9

P.C2.09.02

The role of *lnc-DC* long non-coding RNA and *SOCS1* in the regulation of *STAT3* in coronary artery disease and type 2 diabetes mellitus

A. Alikhah[1], *M. Pahlevan Kakhki*[2], *M. Behmanesh*[1];
[1]*Department of Molecular Genetic, Tarbiat Modares University, Tehran, Iran, Islamic Republic of,* [2]*Department of Clinical Neuroscience, Center for Molecular Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.*

Introduction: Coronary artery disease (CAD) can be classified as an inflammatory disease, which affected by type 2 diabetes mellitus (T2DM). Elevated levels of many inflammatory molecules were found in the serum of patients with CAD. STAT3 molecule as a transcription factor plays an important role in the cytokines expression. Here, we examined the expression levels of *STAT3* and its important regulatory genes *lnc-DC* and *SOCS1*, in patients with CAD and T2DM. Methods: Blood samples were obtained from 37 CAD+ and 36 CAD- patients. These patients were enrolled in this study based on angiography findings and categorized based on T2DM status. The expression levels of *STAT3*, *lnc-DC*, and *SOCS1* genes were examined with Real-time PCR method. Results: A significant increase was observed in the expression of *STAT3* and *lnc-DC* genes but not *SOCS1* in CAD+ versus CAD- patients. These results replicated partially in some groups categorized based on T2DM and CAD status. However, the severity of CAD had no effect on expressions of these genes. Moreover, we found some significant correlations between expressions of *lnc-DC* with *SOCS1* and *STAT3*, which confirmed by in silico analysis. Conclusion: Our results shed further light on the inflammatory aspects of CAD and T2DM with emphasis to JAK/STAT pathway and the regulatory role of long non-coding RNAs in the physiopathology of these diseases.

P.C2.09.03

Modulation of regulatory T cell stability and functions through the T cell receptor signaling molecules Themis1 and Vav1

M. Benamar[1], *R. Marrocco*[1], *I. Bernard*[1], *J. Argenty*[1], *B. Malissen*[2], *R. Lesourne*[1], *A. Saoudi*[1];
[1]*Cptp, Toulouse Cedex 3 FRANCE, France,* [2]*CIML, Aix-Marseille, France.*

Regulatory T cells (Treg) are of paramount importance for restraining excessive immune responses and their manipulation holds enormous therapeutic potential. Our recent results using a congenic rat model suggested that the integrity of Vav1/Themis1 T-cell receptor signaling hub plays a crucial role in Treg lineage stability and suppressive function. Indeed, Themis1 deficiency in BN, but not in LEW rats, led to the development of inflammatory bowel disease (IBD), linked to high levels of IL-17 and IFNg production by Treg, and defects in their suppressive function. Genetic studies revealed that this phenotype depended on a 117 Kb genomic locus, containing the R63W polymorphism on Vav1 that impacted its expression and functions. To test the importance of the Vav1/Themis1 TCR signaling hub in Treg function, we generated Themis1-$T^{-/-}$ mice expressing conditionally Themis1 in thymocytes, but not in peripheral T cells. In contrast to regular germline Themis1 knockout mice, these mice were not lymphopenic and exhibited normal proportions of CD4+ T cells in the thymus and in peripheral lymphoid organs. Next, Themis1-$T^{-/-}$ mice were crossed with $Vav1^{R63W}$ mice to assess the impact of these combined mutations on Treg suppressive functions. Using in vitro approaches, together with in vivo analyses of IBD or melanoma models, we showed that suppressive activity of Treg was impaired in Themis1-deficient mice harboring the mutated Vav1. Functional studies showed the implication of SHP1 in these functional defects. Together, these data showed that Themis1 and Vav1 cooperate in the same signalosome to regulate the suppressive function of regulatory T cells.

P.C2.09.04

Glucocorticoid hormone treatment enhances the cytokine production of regulatory T cells by upregulation of Foxp3 expression

T. Berki*, L. Prenek, E. Ugor, R. Pap, F. Boldizsár, P. Németh;*
Department of Immunology and Biotechnology, University of Pécs, Pécs, Hungary.

Objective: Despite the fact that glucocorticoids (GC) are important therapeutic tools, their effects on regulatory T cells (Treg) are not well defined. The aim of our work was to investigate how GCs influence in vivo the thymic (tTreg) and peripheral Treg (pTreg) differentiation, survival and cytokine production.
Methods: Tregs were detected with flow cytometry in lymphatic organs of 4-6 weeks old BALB/c mice after repeated (2-4 days), high-dose GC treatment using CD4/CD25 cell surface and Foxp3/IL-10/TGFβ/glucocorticoid receptor (GR) intracellular staining. Cytokine, Foxp3, and GR mRNA levels of sorted $CD4^+CD25^{high}$ T cells were analyzed using RT-PCR. Foxp3 and GR localization in Treg cells was investigated with confocal microscopy and FRET analysis.
Results: GC treatment resulted in increased relative tTreg frequency in the thymus, with unchanged absolute cell count. The pTreg ratio and absolute cell count decreased in secondary lymphatic organs. Elevated intracellular IL-10+ and TGFβ+ tTreg and pTreg ratios were measured in GC-treated animals, accompanied with elevated Foxp3 mRNA expression. GC treatment increased TGFβ and IL-35 mRNA in splenic and elevated IL-10 mRNA in thymic tTregs. GC induced nuclear localization of GR in both tTregs and pTregs, which showed high colocalization (~60%) with Foxp3. These data suggest an interaction of these two transcription factors with further increase due to GC treatment in pTregs.
Conclusion: Our data show selective survival of tTregs and elevated production of immunosuppressive cytokines by Treg cells after GC treatment, which may contribute to the immunosuppressive effects of GCs.
Funding: OTKA K105962, GINOP 2.3.2-15-2016-00050.

P.C2.09.05

Expression of PD-1 and PD-L1 markers on T-regulatory cells during cytokine- and anti-CD3-induced proliferation in norm and rheumatoid arthritis

E. A. Blinova*, E. A. Pashkina, D. V. Shevyrev, L. V. Grishina, A. E. Sizikov, V. A. Kozlov;*
Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation.

Rheumatoid arthritis (RA) characterized by disruption of tolerance and Treg dysfunction. The aim of our study was to evaluate expression of functional molecules on Tregs under cytokine-induced proliferation in norm and RA.
The study included 6 patients with RA and 7 healthy donors (average 60±4,4 and 60±4,5 years respectively). After isolation PBMCs were cultivated with or without IL-7 (50ng/ml), IL-15 (50ng/ml) and simultaneously IL-7+IL-15, anti-CD3 antibodies (1mkg/ml) and IL-2 (100ME/ml) during 7days. Analysis of PD-1 and PD-L1 expression on $CD4^+25^+127^{low}$ regulatory cells was performed by flow cytometer CantoII(BD).
Groups of donors and patients didn't differ in number of Tregs and PD-1/PD-L1 expression in peripheral blood. Culturing of PBMCs without stimulation led to a decreasing of Tregs in both groups, and conversely - to an increasing of density of CD25 and CD127, perhaps, due to lack of growth factors. Activation by homeostatic factors promotes the increasing of PD-L1 expression on Tregs, but on the lower level than aCD3+IL-2 stimulation. In RA, after stimulation with IL-7 it was demonstrated more PD-1-positive and less PD-L1-positive Tregs than in norm. Under IL-15 and IL-7+15 stimulation, patients had less $PD\text{-}1^+$ Tregs compared to donors. It was an increase in number of $PD\text{-}L1^+$ Tregs after aCD3+IL-2 stimulation in RA compared to norm.
In RA changes in expression of PD-1/PD-L1 on Tregs under the high doses of IL-7 and IL-15 can influenced on capacity of Tregs to control proliferation of auto-reactive T-cell clones.
The reported study was funded by RFBR and Novosibirsk region, the research project No.17-44-540167.

P.C2.09.06

Identification of VIMP as a novel anti-inflammatory gene of CD4+ effector T cells by a correlation network-guided approach

C. M. Capelle[1,2], *N. Zeng*[1,2], *E. Danilecviciute*[1], *M. Ollert*[1,3], *R. Balling*[4], *F. He*[1];
[1]*Department of Infection and Immunity, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg,* [2]*University of Luxembourg, Esch-sur-Alzette, Luxembourg,* [3]*Odense Research Center for Anaphylaxis (ORCA), Department of Dermatology and Allergy Center, University of Southern Denmark, Odense, Denmark,* [4]*Luxembourg Center for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg.*

Although many key players of the CD4+ T cell inflammatory response have already been identified, many nodes of the underlying network are still missing for a deep understanding of the molecular mechanisms. We previously established a correlation network-guided strategy to identify novel key hub genes and extended the strategy to identify novel genes regulating the inflammatory response of CD4+ effector T cells (Teffs), based on high-resolution time-series data of human Teffs in the first 6 hours following T cell receptor stimulation. We identified VIMP (VCP-interacting protein, also known as SELS, SELENOS), one of the 25 genes encoding the 21st amino acid selenocysteine in humans, as a novel gene involved in Teff function. VIMP is known to be an important component of the endoplasmic reticulum (ER)-associated degradation (ERAD) complex and has functions in cell survival by regulating ER stress. Furthermore, VIMP has been shown to have an anti-inflammatory function in macrophages.
Knocking-down VIMP in Teffs significantly enhanced their proliferation and the expression of different cytokines. By using a computational approach, we predicted the transcription regulatory- and signalling transduction network through which VIMP regulates cytokine expression, based on the dynamic correlation network of Teffs and on transcriptome analysis, and experimentally validated the prediction in a finer scale. Altogether we demonstrate that VIMP inhibits the inflammatory response via both the E2F5 transcription regulatory network and the phosphorylation of NFATC2 through which VIMP regulates the inflammatory response in CD4+ effector T cells.

P.C2.09.07

Lymphatic endothelial cells amongst other stromal cell subsets have unique properties to shape peripheral T cell responses

H. den Braanker[1,2], *A. C. Mus-Otten*[2], *P. S. Asmawidjaja*[2], *N. Davelaar*[2], *A. A. de Smet*[1], *G. P. Akkersdijk*[1], *B. Fioole*[1], *J. B. Jaquet*[1], *A. Hofman*[1], *O. P. Schuitema*[1], *P. A. Bakx*[1], *R. E. Mebius*[3], *S. W. Tas*[4], *H. Yagita*[5], *E. Lubberts*[2], *M. R. Kok*[1], *R. J. Bisoendial*[1];
[1]*Maasstad hospital, Rotterdam, Netherlands,* [2]*Erasmus MC, Rotterdam, Netherlands,* [3]*VUmc, Amsterdam, Netherlands,* [4]*AMC, Amsterdam, Netherlands,* [5]*Juntendo University School of Medicine, Tokyo, Japan.*

Background Psoriatic arthritis is a destructive joint disease developing in approximately 30% of psoriasis patients. Both psoriasis and psoriatic arthritis are T-cell mediated diseases, but how T cells migrate from the skin to joint is unclear. Growing evidence suggests that a defective lymphatic system and the lymphatic endothelial cells (LECs) might play a crucial role in the progression of psoriasis to psoriatic arthritis. **Objective** In this study, we aim to elucidate the role of the lymphatic system in psoriasis and psoriatic arthritis. We hypothesize that healthy LECs have immunoregulatory properties that are lost in psoriatic arthritis allowing transmission of pathogenic T cells from skin to the joints. **Methods** Human dermal LECs ($CD31^+$ $podoplanin^+$) and fibroblasts ($CD31^-$ $podoplanin^-$) were isolated from skin from healthy individuals. Human lymph node LECs ($CD31^+$ $podoplanin^+$) were isolated from patients undergoing vascular surgery. Expression of immunoregulatory molecules was analyzed by flow cytometry and RT-qPCR. **Results** Healthy human dermal LECs and healthy human lymph node LECs express immunoregulatory and costimulatory molecules, such as programmed death-ligand 1 (PD-L1), galectin 9, glucocorticoid-induced TNFR-related ligand (GITRL) and OX40 ligand. Furthermore, both LECs from lymph node and skin as well as skin BECs are $HLA\text{-}DR^+$ in contrast to healthy dermal fibroblasts and healthy synovial fibroblasts. **Conclusion** Both dermal and lymph node LECs are HLA-DR+ and express several immunoregulatory and costimulatory molecules indicating an important role for LECs in shaping peripheral T cell responses. Further studies involving dermal, lymph node and synovial LECs from psoriasis and psoriatic arthritis patients are underway.

P.C2.09.08

IL-17A/F contributes to the pathogenesis of experimental Epidermolysis Bullosa Acquisita

F. Deng[1], *I. Prinz*[2], *X. Yu*[3], *F. Petersen*[4], *R. Ludwig*[5], *C. Hölscher*[1];
[1]*Infection Immunology, Research Centre Borstel, Borstel, Germany,* [2]*Institute for Immunology, Hannover Medical School, Hannover, Germany,* [3]*Autoimmunity of the Lung, Research Centre Borstel, Borstel, Germany,* [4]*Biochemical Immunology, Research Centre Borstel, Borstel, Germany,* [5]*Clinical Dermatology, University Hospital Lübeck, Lübeck, Germany.*

Introduction: Epidermolysis bullosa acquisita (EBA) is a chronic autoimmune skin disease caused by autoantibodies against type VII collagen (ColVII). Anti-ColVII antibodies deposit in dermal-epidermal junction to activate complement and mediate neutrophil infiltration, finally triggering skin fragility and blister formation. Interleukin (IL)-17A and IL-17F, mainly produced by Th17, γδT and innate immune cells, are pro-inflammatory cytokines, and have been reported to mediate infectious and autoimmune diseases by promoting the recruitment and activation of neutrophils. However, the contribution of IL-17A and IL-17F in the pathogenesis of EBA is unclear. **Methods:** Rabbit anti-mColVII IgG was transferred to mice to induce passive systemic EBA model. To elucidate the function of IL-17A and IL-17F in EBA, IL-17A/F-/- and IL-17Ra-/- were examined. IL-17A-eGFP and γδTCR-/- mice were used to determine IL-17A-producing cells. Keratinocytes and fibroblasts were selected for in vitro assays to characterize targets cells of IL-17A in EBA. **Results:** After transfer of anti-mColVII IgG, IL-17A/F-/- and IL-17Ra-/- mice showed a milder disease than C57BL/6 wildtype mice, which was accompanied with lower mRNA levels of IL-6 and CCL2 in skin. Flow cytometry analysis of IL-17A-eGFP mice revealed that γδT cells were the main cell type to produce IL-17A during EBA, which was further confirmed by a disease remission in γδTCR-/- mice. In response to IL-17A, keratinocytes and fibroblasts secreted CXCL-1 in vitro to accelerate neutrophil migration. **Conclusion:** Our results demonstrate that IL-17A/F, derived from γδT cells, plays a pivotal role in EBA by the induction of chemokine release in keratinocytes and fibroblasts to promote neutrophil infiltration.

P.C2.09.09

Relationship between body temperature and lifestyle

K. Kimura*, D.Akiyama;*
Department of Health and Sports Management, Japan University of Economics, Dazaifu, Japan.

Introduction: Maintaining a core temperature of 37.0℃ is important for autoimmunity, but reports in recent years show a declining trend in body temperature in Japan. The aim of this study was to investigate the relationship between tympanic temperature and lifestyle.
Materials and Methods: The subjects were 68 healthy females. The subjects measured their tympanic temperature using a thermometer in the evening. We used a questionnaire format to survey the dietary patterns and activity levels of the subjects. The dietary patterns were assessed by examining the average meal content consumed per week over the last 1-2 months and meal consumption, including nutritional content and other factors. The activity levels represented the average duration of physical activity per day and exercise duration per week (4 intensity levels: 3≤ metabolic equivalents (METs) <4, 4≤ METs <6, 6≤ METs <8, 8 ≤ METs ≤15). Correlation coefficient was calculated to determine correlations between tympanic temperature and each item.
Results: There were negative correlations between tympanic temperature and consumption of fat, saturated fatty acid and monounsaturated fatty acid. 36 subjects had tympanic temperature of 36.5℃ or more (normal temperature, N), and 32 subjects had temperatures of less than 36.5℃ (low temperature, L). We compared each item on either side of 36.5℃, the total caloric intake, consumption of saturated fatty acid and monounsaturated fatty acid were significantly lower for N.
Conclusions: This study showed that there was a significant correlation between tympanic temperature and consumption of fat, saturated fatty acid and monounsaturated fatty acid.

P.C2.09.10

Treg specific constitutive Nrf2 activation precipitates an inflammatory state

P. Klemm[1], *B. Denecke*[2], *A. Schippers*[1], *N. Wagner*[1], *K. Tenbrock*[1], *K. Ohl*[1];
[1]*Department of Pediatrics, University hospital RWTH Aachen, Germany,* [2]*Interdisciplinary Centre for Clinical Research (IZKF), University hospital RWTH Aachen, Germany.*

Immune cells are constantly confronted with intracellular and extracellular radical oxygen species (ROS) under steady-state and even more under inflammatory and pathogenic conditions. To investigate the effects of oxidative stress and ROS molecules in regulatory T cells (T_{regs}), we deciphered the role of Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) in this context. T_{regs} were already found to be more resistant to ROS than effector T cells and activated T_{reg} cells show higher expression of genes, which belong to the Nrf2-mediated oxidative stress response compared to activated effector T cells. Here we report a previously unrecognized negative role of Nrf2 in T_{regs}. While mice bearing a constitutive activation of Nrf2 in all immune cells ($Vav^{cre}Keap^{fl/fl}$) accumulate high percentages of Foxp3-positive T_{regs} in the spleen, lymph nodes and thymus, their suppressive capacity seems to be defective. Interestingly, a T_{reg} specific activation of Nrf2 ($Foxp3^{cre}Keap^{fl/fl}$) results in an auto-inflammatory phenotype with immune cell infiltrates in the lung, enhanced effector T cell activation and high percentages of IFN-γ producing effector T cells. Moreover, the constitutive Nrf2 activation in T_{regs} increases their *in-vitro* proliferation, glucose uptake and mTOR activity, while the differentiation and Foxp3 expression in T_{regs} declines. We demonstrate for the first time that constitutive Nrf2 activation specific to T_{regs} affects T_{reg} lineage stability and metabolism and might thereby induce an auto-inflammatory phenotype. Thus, our results may have implications for diseases associated with oxidative stress and dysregulated T_{reg} responses.

P.C2.09.11

The role of $CD8^+$ T lymphocytes in experimental nephrotoxic serum nephritis

A. A. Mooslechner*, A. H. Kirsch, K. Artinger, I. Aringer, F. Moschovaki-Filippidou, C. Schabhüttl, K. Schweighofer, A. R. Rosenkranz, K. Eller;*
Clinical Division of Nephrology, Graz, Austria.

Nephrotoxic serum nephritis (NTS) is known as a $CD4^+$ T cell-dependent murine model of anti-glomerular basement membrane disease. Although the presence of $CD8^+$ T cells in disease progression is known, their role in its pathology is not clear. Recent controversial findings challenge our knowledge of $CD8^+$ T cells and their function solely as effector T cells by suggesting the existence of a regulatory subset, characterized by the marker CD122. We here aim to understand the role of $CD8^+$ T cells and its subset of CD8CD122 double positives in progression of NTS.
Male C57Bl/6J mice, 8 weeks of age, were either treated with an anti-CD8α and/or anti-CD122 depleting antibody, or respective isotype control, one day before induction of NTS and were followed for 5 or 21 days. Renal endpoints such as albuminuria, blood urea nitrogen (BUN), inflammatory cell infiltration and glomerulosclerosis were assessed.
Successful depletion of $CD8^+$ and $CD122^+$ lymphocytes was observed by flow cytometry and confirmed by immunohistochemistry. At disease onset, mice treated with anti-CD8α scored lower levels of BUN. Mice treated with anti-CD122 or both antibodies showed no changes in renal endpoints. After 21 days of disease progression, the group treated with both antibodies scored significantly higher levels of BUN as well as albuminuria/creatinine ratio.
Our data support the hypothesis that $CD8^+$ T lymphocytes play a crucial role in immune regulation of NTS. However, our findings suggest a more complex picture with distinct roles for subpopulations of these lymphocytes, which were formerly thought to be effector T cells only.

P.C2.09.12

PUTRID: a marker that identifies pure and stable regulatory T cells

R. Opstelten[1], *E. Cuadrado*[1], *S. de Kivit*[2], *M. C. Slot*[1], *M. van den Biggelaar*[1], *A. M. Scott*[3], *J. Borst*[2], *D. Amsen*[1];
[1]*Sanquin Research, Amsterdam, Netherlands,* [2]*Netherlands Cancer Institute, Amsterdam, Netherlands,* [3]*Olivia Newton-John Cancer Research Institute, Melbourne, Australia.*

Regulatory T-cells (Tregs) can alleviate autoimmune and organ transplant-related diseases through adoptive cellular therapy. There are two different sources of Tregs. Thymic-derived Tregs are stably committed to the Treg lineage. Tregs that are induced to differentiate from conventional T-cells can, however, lose their suppressive capacity and start producing effector cytokines, thereby forming a potential danger when given to a patient. Discrimination between these lineages is currently not possible. Here, we report the identification of the immunoglobulin superfamily member PUTRID as a surface marker that identifies stable human Tregs. When freshly isolated, $PUTRID^{high}$ Tregs express the key Treg transcription factors FoxP3 and Helios and are unable to produce effector cytokines upon stimulation. Their $FoxP3^+Helios^+PUTRID^{high}$ phenotype is maintained in culture, whereas their $PUTRID^{low}$ counterparts do not stably express these critical molecules. $PUTRID^{high}$ Tregs are suppressive, have a fully demethylated FoxP3 promotor and remain unresponsive to cytokine-inducing stimuli after expansion. High expression of PUTRID is found on both naïve and effector Tregs. In this latter population, $PUTRID^{high}$ cells are the only subset capable of proliferation. Our findings thus reveal previously unrecognized functional heterogeneity among human Tregs. Importantly, our results demonstrate a strategy to isolate Tregs for safer and more efficacious adoptive cellular therapy.

P.C2.09.13

USING CRISPR-CAS9 TO ELUCIDATE THE ROLE OF TNF-RECEPTORS IN TREG FUNCTION

A. M. Pesenacker[1,2,3], *U. Nguyen*[1,2], *J. Gillies*[1,2], *M. K. Levings*[1,2];
[1]*BC Children's Hospital Research Institute, Vancouver, Canada,* [2]*University of British Columbia, Vancouver, Canada,* [3]*Institute of Immunity and Transplantation, UCL Royal Free Hospital, London, United Kingdom.*

FOXP3+ regulatory T cells (Tregs) are crucial to maintain immune tolerance and prevent autoimmune diseases such as Juvenile idiopathic arthritis (JIA) and type 1 diabetes (T1D). Treg function, however, can be influenced by the environment of inflammation. For example, Tumour necrosis factor alpha (TNF-), which is abundant in inflamed JIA joints, can have positive or negative effects on Treg function. TNF- has two receptors, CD120a and CD120b, with CD120a linked to cell death and CD120b to survival. We found skewed CD120a to CD120b expression on Tregs in the inflamed JIA joints, with increased CD120a and decreased CD120b levels. We hypothesized that this altered CD120a:CD120b expression ratios would affect TNF--driven changes in Treg function. To elucidate the functional relevance of the TNF-receptors in primary human Tregs, CRISPR-Cas9 methodology was used to knockout (KO) CD120b, or CD120a or CD120b were over-expressed using lentiviral transduction. CD120bKO or CD120a-overexpressing Tregs possessed a normal Treg phenotype, with expression of FOXP3 and demethylated TSDR, but had higher levels of apoptosis, expression of exhaustion markers (PD-1, Lag3), and decreased suppressive capacity. Upon TCR plus TNF- stimulation only CD120b-expressing Tregs had increased expression of FOXP3, activation markers and proliferation compared to TCR stimulus alone. Understanding how Treg function is controlled by TNF- may reveal mechanisms which lead to the failure of immune tolerance in the joint, provide insight into uncharacterized effects of TNF- blockade on Tregs and ultimately to new therapeutic approaches to restore immune regulation in autoimmunity.

P.C2.09.14

CXCR3+ CD45RA- T cells as downstream effectors of tissue inflammation in psoriatic arthritis

F. Casciano[1], *M. Diani*[2], *L. Marongiu*[3], *M. Longhi*[2], *A. Altomare*[2], *P. Secchiero*[4], *R. Gambari*[5], *F. Granucci*[3], ***E. Reali***[2,5];
[1]*Department of Morphology, Surgery and Experimental Medicine and LTTA Centre, University of Ferrara,, Ferrara, Italy,* [2]*IRCCS Istituto Ortopedico Galeazzi, Milan, Italy,* [3]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy,* [4]*Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Italy,* [5]*Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy.*

Little is known about the link between T cell responses arising in the skin and systemic and joint inflammation in patients with Psoriatic arthritis (PsA). The aim of this study is to explore the T cell recirculating subsets as possible pathogenic elements in systemic and joint manifestations of PsA.
We performed an extensive analysis of the phenotype and cytokine expression in circulating $CD4^+$ and $CD8^+$ T cells in a cohort 69 subjects including 15 patients with psoriatic arthritis (PsA), 28 patients with cutaneous psoriasis and 26 healthy subjects. For each subset correlation was calculated with the serum level of C-reactive protein. In selected patients with PsA we also performed a parallel analysis by comparing T cells in blood and synovial fluid.
In the circulation of PsA patients, we found a marked decrease in the percentage of $CXCR3^+CD45RA^-$ memory T cells, both in the $CD4^+$ and $CD8^+$ compartments. Consistently, there was a lower level of IFNγ-producing $CD4^+$ $CD45RA^-$ T cells in the blood of patients with PsA.
By contrast in the synovial fluid, we found a strong enhancement of $CXCR3^+$ and IFNγ-producing $CD4^+$ and $CD8^+$ T cells.
In the circulation of PsA patients IL-17-producing $CD4^+$ and $CD8^+CD45RA^-$ T cells were significantly more abundant and $CCR6^+CD4^+$ T effector memory cells positively correlated with the extent of systemic inflammation.
The results enlighten the role of $CXCR3^+$ IFNγ-producing effector cell recruitment from the blood stream to the inflamed tissue as a downstream mechanism in the pathogenesis of PsA.
Grant from Fondazione Natalino Corazza Onlus.

P.C2.09.15

The role of cMAF and IKZF3 in anti-TNF induced IL-10 expression in CD4+ T cells

M. L. Ridley[1], *V. Fleskens*[1], *C. A. Roberts*[1], *S. Lalanunhlimi*[1], *G. A. Povoleri*[1], *P. Lavender*[2], *L. S. Taams*[1];
[1]*Centre for Inflammation Biology and Cancer Immunology (CIBCI), Dept Inflammation Biology, School of Immunology & Microbial Sciences, King's College London, London, United Kingdom,* [2]*MRC and Asthma UK Centre in Allergic Mechanisms of Asthma, School of Immunology & Microbial Sciences, King's College London, London, United Kingdom.*

We have previously shown that anti-TNF treatment increases the frequency of IL-10 producing CD4+ T cells *in vitro* and *ex vivo*. We investigated whether transcriptional regulation of IL-10 expression following TNF-blockade involved the transcription factors IKZF3 and cMAF.
We examined the expression of IL-10, IKZF3 and cMAF through flow cytometry and quantitative-PCR. We manipulated IKZF3 expression using lentiviral overexpression and pharmacological inhibition.
IL-10 expression increased in CD4+ T cells upon stimulation, but was maintained at higher levels at the mRNA and protein level upon culture with anti-TNF after 3 days. cMAF was expressed by IL-10+ CD4+ T cells but was not significantly changed by anti-TNF. IKZF3 was expressed at higher levels in anti-TNF treated IL-10+ CD4+ T cells compared to other cytokine producing cells. Pharmacological inhibition of IKZF3 using the drug lenalidomide significantly reduced the frequencies of cells expressing IL-10. However, lentiviral over-expression of IKZF3 was not sufficient to induce *IL10* mRNA expression. Luciferase reporter assays using putative regulatory regions of the *IL10* locus are ongoing to investigate the transcriptional regulation of *IL10* by cMAF and IKZF3.
Our findings indicate that the increased frequency of IL-10 producing CD4+ T cells after anti-TNF treatment is not due to altered cMAF expression. Our findings also indicate that although there is an association between IKZF3 and IL-10 expression upon TNF blockade, IKZF3 by itself is not sufficient to drive the expression of *IL10* in CD4+ T cells. Funded by Arthritis Research UK (Ref 21139)

P.C2.09.16

Thymus derived Treg cell development is regulated by C type lectin mediated BIC miRNA155 expression

R. Sanchez Diaz, *R. Blanco Dominguez, B. Linillos Pradillo, M. Relaño, P. Martin Fernandez;*
CNIC, Madrid, Spain.

Normal 0 false false false EN-US X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin-top:0in; mso-para-margin-right:0in; mso-para-margin-bottom:8.0pt; mso-para-margin-left:0in; line-height:107%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:"Calibri",sans-serif; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin;} Thymus-derived regulatory T (tTreg) cells are key to preventing autoimmune diseases, but the mechanisms involved in their development remain unsolved. Here, we show that the C-type lectin receptor CD69 controls tTreg cell development and peripheral Treg cell homeostasis through the regulation of BIC microRNA 155 (miR155) and its target, suppressor of cytokine signaling 1 (SOCS1). Using Foxp3 mRFP*cd69_/_* or Foxp3mRFP*cd69_/_* reporter mice and short hairpin RNA (shRNA) mediated silencing and miR155 transfection approaches, we found that CD69 deficiency impaired the signal transducer and activator of transcription 5 (STAT5) pathway in Foxp3 cells. This results in BIC miR155 inhibition, increased SOCS1 expression, and severely impaired tTreg cell development in embryos, adults, and Rag2_/_γ c_/_ hematopoietic chimeras reconstituted with *cd69_/_* stem cells. Accordingly, *mirn155_/_* mice have an impaired development of CD69 tTreg cells and overexpression of the miR155 induced CD69 pathway, suggesting that both molecules might be concomitantly activated in a positive-feedback loop. Moreover, *in vitro*-inducible CD25 Treg (iTreg) cell development is inhibited in *Il2r_/_/cd69_/_* mice. Our data highlight the contribution of CD69 as a nonredundant key regulator of BIC miR155 dependent Treg cell development and homeostasis.

P.C2.09.17

Serum cytokines and biological therapy of psoriasis -Prospects for personalized treatment?

S. M. Solberg[1,2,3], *L. F. Sandvik*[4,5], *M. Eidsheim*[1], *R. Jonsson*[1,3], *Y. T. Bryceson*[1,3,6], *S. Appel*[1,3];
[1]*Broegelmann Research Laboratory, Bergen, Norway,* [2]*Department of Dermatology, Haukeland University Hospital, Bergen, Norway,* [3]*Department of Clinical Science, University of Bergen, Norway,* [4]*Department of Dermatology, Haukeland University Hospital, Bergen, Norway,* [5]*Department of Clinical Medicine, University of Bergen, Bergen, Norway,* [6]*Centre for Hematology and Regenerative Medicine, Department of Medicine, Karolinska Institutet, Sweden.*

Introduction. Psoriasis is an immune mediated disease where the IL-23/Th17 axis and TNF are central. The aim was to compare serum cytokines in psoriasis patients receiving biological therapy, to Psoriasis Area and Severity Index (PASI) and Dermatological Life Quality Index (DLQI).
Methods. Clinical parameters and 25 serum cytokines (determined by Luminex technology) were measured in 40 patients at inclusion and after 3-4 months.
Results. PASI and DLQI decreased by 71.03% and 64.87%, respectively. Logistic regression revealed four times higher risk of severe psoriasis when IL-17A increased by 1pg/mL (OR 4.064, CI: 1.008-16.383, $p < 0$ .049). Significant association between PASI90 and increase of IL-5, IL-10, IL-12, IL-22 and GM-CSF was detected. Fold change of IL-2 correlated positively with PASI and DLQI improvement. BMI correlated with TNF, IL-22 and IL-1RA.
Conclusions. The important role of IL-17A in psoriasis is confirmed by this study. Increase of GM-CSF, IL-5, IL-10, IL-12, IL-22 and IL-2 during follow-up, correlated with treatment effect. Serum cytokines might be useful biomarkers for monitoring disease activity and optimizing therapeutic strategies for psoriasis patients.
Grant: Norwegian association for Dermatology and Venerology sponsored the ProcartaPlex kit. Fundings: Broegelmann Foundation and Western Norwegian Health authorities.

P.C2.09.19

Myeloid-derived suppressor cells in children with severe psoriasis

*D. Gerasimova[1], T. Radygina[1], N. Murashkin[1], **A. Toptygina**[2,3], S. Petrichuk[1];*
[1]Federal State Autonomous Institution "National Medical Research Center of Children's Health", Moscow, Russian Federation, [2]G.N.Gabrichevsky Research Institute for Epidemiology and Microbiology, Moscow, Russian Federation, [3]Lomonosov Moscow State University, Moscow, Russian Federation.

Psoriasis is a chronic immune-mediated inflammatory skin disease. Lately, participation in the pathogenesis of psoriasis of myeloid-derived suppressor cells (MDSCs) and Tregs cells has been studied. The goal was to determine the number of MDSCs in the blood of children with psoriasis. 78 children with psoriasis at the age between 0.5-18 years were examined: 46 primary patients and 32 children on treatment with a TNF-α inhibitor. The severity of psoriasis was assessed by PASI. The control group consisted of 31 children. The number of MDSCs and the indicator of cell mediated immunity (Tregs, act-Th and Th17) were determined on the NovoCyte ACEA flow cytometr. The group of children with psoriasis was characterized by a large spread of the number of MDSCs (6-198 cells/ml) relative to the control group (10-54 cells/ml, F = 4.6, p <0.01). The number of MDSCs is characterized by a phase dependence on the duration of the disease and does not depend on the age of the patients. During the first 5 years of the disease MDSCs amount increases but after 6 years of disease their number decreases by 30%. With an increasing of the disease duration, amount of Th17 cells increase (R=0.4) while amount of the Tregs cells decrease (R= - 0.39). Among children with severe psoriasis of MDSCs increases with increasing of PASI. As the disease progresses, the amount of MDSCs decreases but the amount of Th17 and act-Th increases.

P.C2.10 Immune signaling and therapy in autoimmunity - Part 10

P.C2.10.01

Changes in cytokine profiles of rheumatoid arthritis patients after Adalimumab or Etanercept anti TNFα therapy.

***T. Ahmed**;*
Ulster University, Londonderry, United Kingdom.

Rheumatoid arthritis (RA) affects approximately 1% of the population and without effective treatment can lead to permanent disability. Biological drugs Adalimumab (ADL) and Etanercept (ETN) target tumour necrosis factor α (TNFα), achieving ~70% response rates. These drugs are expensive and therefore it is imperative to stratify patients who will receive clinical benefit. Here we report that whilst both ADL and ETN inhibit TNFα activity; they produce distinct changes in blood cytokine levels. Anti-TNF naive RA patients were treated with ADL or ETN. Serum samples were collected at baseline (T0) and 6 months post therapy (T6). Serum cytokines IFNγ, IL-10, IL-12 p70, IL-13, IL-4, IL-6, IL-8 and TNFα were measured by MesoScale assay. Cytokine concentrations are reported in pg/mL and statistical significance was determined using Wilcoxon matched pairs signed rank test. After 6 months of therapy; ADL treated patients had significantly lower concentration of IL-6 (T0=3.03 vs T6=0.74), IL-8 (T0=14.18 vs 11.35) and TNFα (1.96 vs 1.704) compared to baseline. ETN treated patients had a significantly higher concentration of IL-10 (T0=0.35 vs 0.60) and TNFα (T0=1.64 vs 52.23). We report that ADL and ETN have differing effects upon blood cytokines. ADL reduced IL-6, IL-8 and TNFα. ETN elevated IL-10 and TNFα. From 11 ETN treated patients; 9 were good responders, 1 was moderate responder and 1 was non-responder according to EULAR classification. TNFα-ETN complexes may have increased blood half-life & reduced clearance, thus explaining why TNFα is elevated in these patients; the majority of whom are responding to treatment.

P.C2.10.02

Sex-based differences in association between circulating T cell subsets and disease activity in untreated early rheumatoid arthritis patients

***J. Aldridge**[1], J. M. Pandya[1], L. Meurs[1], K. Andersson[1], I. Nordström[1], E. Theander[2], A. Lundell[1], A. H. Rudin[1];*
[1]Dept of Rheumatology and Inflammation Research, Institute of Medicine, Gothenburg, Sweden, [2]Dept of Rheumatology, Lund University, Skåne, Lund, Sweden.

Introduction: It is not known if sex-based disparities in immunological factors contribute to the disease process in rheumatoid arthritis (RA). We examined whether circulating T cell subset proportions and their association with disease activity differed in male and female patients with untreated early rheumatoid arthritis (ueRA). **Methods**: Proportions of T cell subsets were analyzed in peripheral blood from 70 ueRA DMARD- and corticosteroid-naïve patients (50 females and 20 males) and in 31 healthy age- and sex-matched controls. Broad analysis of helper and regulatory $CD4^+$ T cell subsets was done using flow cytometry. Disease activity in patients was assessed using DAS28, CDAI, swollen joint counts, tender joint counts, CRP and ESR. **Results**: In male, but not female, ueRA patients Th2 cells showed a positive association with disease activity and correlated significantly with DAS28-ESR, CDAI and tender joint counts. Likewise, proportions of non-regulatory $CTLA\text{-}4^+$ T cells associated positively with disease activity in male patients only, and correlated with DAS28-ESR. In contrast, there was a negative relation between Th1Th17 subset proportions and disease activity in males only. Proportions of Th1 and Th17 cells showed no relation to disease activity in either males or females. There were no significant differences in proportions of T cell subsets between the sexes in patients with ueRA. **Conclusion**: Our findings show sex-based differences in the association between T cell subsets and disease activity in ueRA patients Moreover, Th2 helper T cells may have a role in regulating disease activity in male patients.

P.C2.10.03

Sucrose octasulphate inhibits the differentiation of monocytes to TNF-α synthesising macrophages through inhibition of the PKC pathway: explanation for its anti-rheumatic activity

***P. Bajwa**[1], N. Garrido-Mesa[1], M. P. Seed[2], S. S. Ayoub[2];*
[1]Medicines Research Group, School of Health Sport and Bioscience, University of East London, Stratford, United Kingdom, [2]Clinical Research Group, School of Health Sport and Bioscience, University of East London, Stratford, United Kingdom.

Rheumatoid arthritis, is, driven by an array of cytokines such as tumour necrosis factor-α (TNF-α) synthesised by T-cells and macrophages. Sulphated disaccharides (SDS) are released by heparanase during inflammation to inhibit T-cell function. These, including the synthetic analogue sucrose octasulphate (SOS) inhibit rodent models of arthritis and reduce T-cell function. We have shown SOS inhibits monocyte-to-macrophage (Mϕ) differentiation. The current study was conducted to explore possible mechanisms.
Human monocytes U937 were pre-treated with SOS (10^{-11} to 10^{-4} M) 2h prior to the induction of differentiation by 8nM phorbol 12-myristate 13-acetate (PMA). The cells were then washed and re-suspended in fresh medium for 24h followed by 1μg/ml LPS for 6h to induce TNF-α synthesis. The action of SOS on monocyte differentiation, expression of Mϕ cell surface markers, TNF-α synthesis, intracellular calcium mobilisation (marker for PKC activation) and activation of ERK1/2, p38MAPKinases were investigated.
SOS inhibited differentiation (cell adhesion), down-regulated CD14, CD11a, CD11b and CD68 expression by PMA stimulated U937 cells and reduced LPS induced TNF-α synthesis by the residual macrophages. The inhibitory action of SOS on monocyte differentiation was associated with the down-regulation of intracellular calcium mobilisation and phosphorylation of the ERK1/2, and p38MAPK cell signalling pathways indicative of inhibition of PKC cell signalling.
SOS may mediate its anti-rheumatic actions through the inhibition of pathways downstream of PKC. SDS are released from tissue heparans during inflammation, and we hypothesise that synthetic SDS analogues may have potential as a new basis for treating chronic inflammatory disease such as rheumatoid arthritis.

P.C2.10.04

Dynamics of circulating TNF during adalimumab treatment of rheumatoid arthritis using a novel drug-tolerant TNF assay

***L. C. Berkhout**[1], M. J. l'Ami[2], J. Ruwaard[2], M. H. Hart[1], P. Ooijevaar-de Heer[1], K. Bloem[1], M. T. Nurmohamed[2,3], R. F. van Vollenhoven[2,3,4], M. Boers[3,5], C. H. Smith[6], G. Wolbink[1], T. Rispens[1];*
[1]Department of Immunopathology, Sanquin Research, Amsterdam, Netherlands, [2]Amsterdam Rheumatology and immunology Center | Reade, Amsterdam, Netherlands, [3]Amsterdam Rheumatology and immunology Center | VU University Medical Center, Amsterdam, Netherlands, [4]Amsterdam Rheumatology and immunology Center | Academic Medical Center, Amsterdam, Netherlands, [5]Department of Epidemiology & Biostatistics, VU University Medical Center, Amsterdam, Netherlands, [6]St. John's Institute of Dermatology, Division of Genetics and Molecular Medicine, Kings College London, London, United Kingdom.

Rheumatoid arthritis (RA) patients can be successfully treated with tumor necrosis factor- (TNF) inhibitors, including adalimumab, which binds TNF to form inactive complexes. Once in remission, a proportion of patients can successfully discontinue adalimumab treatment, indicating that blocking TNF is no longer required for disease control. We developed a novel competition assay that can quantify TNF in the presence of large amounts of TNF-inhibitor, i.e. a 'drug-tolerant' assay. This assay was used to quantify TNF levels on initiation and during 2 years of adalimumab treatment in 193 consecutive RA patients. We investigated, for the first time, the relationship between TNF levels and clinical response.
Circulating TNF levels were close to the detection limit at baseline, increased on average >50-fold upon adalimumab treatment, and reached a stable level in time in the majority of patients, regardless of disease activity. During treatment, TNF was in complex with adalimumab, and recovered as inactive 3:1 adalimumab:TNF complexes. Remarkably, low TNF levels at week four were associated with a significantly higher frequency of anti-drug antibodies at subsequent time points, significantly less methotrexate use at baseline, and less frequent remission after 52 weeks.
In conclusion, longitudinal TNF levels are mostly stable in time during adalimumab treatment, and may therefore not predict successful dose-interval prolongation or treatment discontinuation. However, low TNF levels in the early phase of treatment (week 4) are strongly associated with anti-drug antibody formation and may be used as timely predictor of non-response towards adalimumab treatment.
Grant 436001001, by ZonMw in the program 2Treat

P.C2.10.05

Effects of the medications anakinra and tocilizumab on CD8+iTregs differentiation and function

*M. L. Kristensen[1], **U. Bjarnadóttir**[2];*
[1]Department of Immunology, Landspitali - University Hospital, Reykjavik, Iceland, [2]Department of Immunology, Landspitali - University Hospital. Reykjavík, Iceland, Reykjavik, Iceland.

Introduction: Regulatory CD8$^+$ T-cells (CD8$^+$iTregs) may play an important role in preventing autoimmune processes in the body by their suppressive function. Anakinra (anti-IL-1β mAb) and tocilizumab (anti-IL-6 receptor mAb) are used for the treatment of autoimmune diseases such as RA.
Aim: The aim of this study was to evaluate the role of IL-6 and IL-1β on the differentiation and function of CD8$^+$ iTregs.
Material and methods: Naïve human CD8$^+$CD45RA$^+$ T cells were stimulated with αCD3/αCD28 in the presence of IL-2/TGF-β1, with/without αIL-6 (0.1/10/1000μg/mL) and αIL-1β (0.5/5/500μg/mL) for 120 hrs. Three subpopulations were defined as CD8$^+$/CD127$^-$/CD25$^+$/FoxP3$^{int/hi}$, CD25$^+$FoxP3$^-$ and CD25$^-$FoxP3^{int} and their cytokine pattern observed (IL-17, IFNγ, IL-6, IL-9 and PD1).
Results: CD8$^+$ iTregs were significantly induced and defined as CD8$^+$/CD127$^-$/CD25$^+$/FoxP3$^{int/hi}$. Both αIL-6 (p=0.002) and αIL-1β (p=0.016) inhibited their induction. Anti-IL-6 treatment reduced both PD1 and IL-9 cellular CD8$^+$iTregs expression. The induction of iTregs in the presence of IL-2 and TGF-β1 resulted in an increased secretion of IL-21**, IL-22**, IL-9**, IL-18* and IL-13**. However, anti-IL-6 treatment inhibited the secretion of IL-6**, IL-21** and IL-22**, whereas, only IL-6* and IL-1β** were found to be reduced following anti-IL-1β treatment during the induction of CD8$^+$ iTregs.
Conclusion: CD8$^+$ iTregs induction is dependent on IL-6 and is possibly driven through an IL-9 and PD1 dependent intracellular mechanism. However, the role of IL-1β on CD8+iTregs may be driven through IL-6 induction. Both IL-21 and IL-22 secretion are reduced following tocilizumab treatment during in vitro stimulation of CD8$^+$ T-cells, suggesting a therapeutic role in a B-cell driven autoimmunity.

P.C2.10.08

Different patterns of circulating anti-ENA specific antibody secreting cells and/or ENA-specific B-lymphocytes are detected in SLE patients

***R. de la Varga Martínez**[1,2], B. Rodríguez Bayona[3], G. A. Áñez Sturchio[4], F. Medina Varo[4], C. Rodríguez[5];*
[1]Servicio de Inmunología. UGC de Laboratorios Clínicos. Hospital Universitario Virgen del Rocío, Sevilla, Spain, [2]Unidad de Investigación y Servicio de Inmunología. Hospital Universitario Puerta del Mar, Cádiz, Spain, [3]Área de Inmunología. UGC de Análisis Clínicos. Hospital Juan Ramón Jiménez, Huelva, Spain, [4]Unidad de Reumatología, UGC de Cirugía Ortopédica, Traumatología y Reumatología. Hospital Universitario Puerta del Mar, Cádiz, Spain, [5]Servicio de Inmunología, UGC de Hematología, Inmunología y Genética. Hospital Universitario Puerta del Mar, Cádiz, Spain.

Introduction: Autoantibody (Ab) secretion can be maintained by long-lived Ab-secreting cells (ASC) and by differentiation of auto-reactive B-Lymphocytes (ABL). However, their relative contribution to serum Ab production in SLE is not completely known. Objective: To detect ENA-specific ABL (ENA-ABL) and ASC producing anti-ENA Ab (ENA-ASC) and to analyse their relationship to anti-ENA Ab levels in serum in SLE patients. Material and methods: Blood samples from 20 SLE patients with serum anti-ENA Ab were studied. ENA-ABL were purified by immunoselection with ENA-bearing immunobeads (QUANTA-Flash®-ENA7, Werfen). ENA-ABL were stimulated in culture with anti-CD40 mAb plus IL-21 and BAFF and anti-ENA Ab secretion into the supernatants was measured. ENA-ASC were identified as cells capable to produce anti-ENA Ab spontaneously in culture. In serum and cultures, anti-ENA Ab and IgG were determined by chemiluminescence and ELISA, respectively. Results: ENA-ABL and ENA-ASC were detected in 15 and 10 patients, respectively. Four different profiles were observed. A majority of patients (8/20) showed a predominant ABL profile. A main ASC response was seen in 3 patients. A mixed profile, with both ABL and ASC, was detected in 3 patients. Finally, a negative profile characterised by non-appreciable ABL and/or ASC was seen in 6 patients. Conclusions: ENA-specific ABL and ASC could be responsible for serum anti-ENA Ab levels. Diverse profiles of ABL and/or ASC predominance were observed. Tracking auto-reactive ASC and ABL can represent a biomarker for using therapies blocking B-cells or ASC and a new tool for monitoring minimal residual autoimmune disease in SLE patients.

P.C2.10.09

Toll-like receptor 9 influences inflammatory arthritis and osteoclastogenesis

***A. Fischer**[1], S. Abdollahi-Rodsaz[2], A. Yau[3], E. Lönnblom[3], R. Holmdahl[3], G. Steiner[1];*
[1]Department of Rheumatology, Medical University of Vienna, Vienna, Austria, [2]Department of Rheumatology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [3]Medical Inflammation Research, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden.

Introduction. Release and insufficient removal of endogenous nucleic acids may trigger autoimmune reactions implicated in initiating systemic autoimmune diseases including rheumatoid arthritis (RA). The DNA sensing Toll-like receptor (TLR) 9 has been linked to pathogenic autoimmunity but its role in RA is unclear. Therefore, we investigated the role of TLR9 in in rats with pristane-induced arthritis (PIA) and in mice with streptococcal cell wall (SCW) induced arthritis. **Methods**. Rats were prophylactically treated with a TLR9 antagonist while SCW arthritis was induced in TLR9$^{-/-}$ mice. Arthritis was scored using established scoring systems, inflammation and bone erosions were quantified by histomorphometry. Serum levels of α-1-acid-glycoprotein (AGP), rheumatoid factor (RF) and IL-6 were analyzed by ELISA. Furthermore, the role of TLR9 in osteoclast differentiation was investigated *in vitro*. **Results**. In PIA, the treatment with the TLR9 antagonist led to significantly reduced inflammation, bone erosion and cartilage degradation accompanied by a reduction of AGP, IL-6 and RF. Moreover, the T cell-dependent phase of SCW arthritis was significantly suppressed in TLR9$^{-/-}$ mice. Remarkably, TLR7 and TLR9 mRNA levels differed in the course of *in vitro* osteoclastogenesis. Whereas TLR7 expression remained constant throughout osteoclastogenesis, expression of TLR9 was higher in precursor cells than in mature osteoclasts and stimulation with a TLR9 agonist (CpG) completely inhibited osteoclastogenesis. **Conclusions**. Taken together, the results suggest a role for TLR9 in the T cell-dependent phases of PIA and SCW arthritis and thus an important involvement of TLR9 in the initiation of autoimmune arthritis and during osteoclastogenesis.

P.C2.10.10

Osteoclast progenitors expressing CCR2 are expanded in collagen-induced arthritis and may be involved in bone resorption intensity

***D. Flegar**[1,2], A. Sucur[1,2], A. Markotic[1,2], N. Lukac[3,2], N. Kovacic[3,2], T. Kelava[1,2], V. Katavic[3,2], K. Zrinski Petrovic[3,2], D. Grcevic[1,2];*
[1]Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia, [2]Laboratory for Molecular Immunology, Croatian Institute for Brain Research, Zagreb, Croatia, [3]Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia.

Introduction: Osteoclast progenitors (OCP) originate from myeloid lineage and mature to osteoclasts, specialized bone resorbing cells. Osteoclast mediated bone and joint destruction is characteristic for rheumatoid arthritis (RA). We investigated frequencies of different OCP subsets and their osteoclastogenic potential in mice with collagen-induced arthritis (CIA), a mouse RA model.
Methods: After receiving Ethical approval, C57BL/6 and DBA mice were immunized with chicken type II collagen to induce CIA, which was confirmed by micro-CT, histology and serum CTX levels. Distal tibia bone marrow (BM) cells were immunophenotyped for hematopoietic markers and chemokine receptor expression. Sorted OCP subsets were assessed for proliferative response by CFSE and TRAP+ osteoclasts number by culturing with M-CSF/RANKL. Mice developing CIA (day 15-23 after immunization) were treated *in vivo* with methotrexate (2mg/kg) and CCR2 receptor antagonist (CRA) (4mg/kg) to assess effect on OCP frequency.
Results: Arthritic mice showed histological presence of BM osteitis and bone destruction assessed by micro-CT and CTX levels. Frequency of CD45+B220-CD3-NK1.1-Ly6G-CD11b−/loCD115+ OCPs was significantly increased in CIA (54% vs. 26% in control), with specific expansion of CCR2+ subset. Regarding the CCR2 expression level, CCR2lo subset underwent through more divisions and generated multinucleated TRAP+ osteoclasts more efficiently, whereas CCR2hi subset generated osteoclasts only when cultured at high density. Our preliminary results indicate that combined methotrexate/CRA treatment may affect frequency of OCPs.
Conclusion: OCP subset expressing CCR2 may contribute to bone resorption in arthritis. Therefore, inhibition of CCL2/CCR2 signaling presents a new therapeutic approach to reduce osteoclast activity.
Support: Croatian Science Foundation project 5699.

P.C2.10.11

HISTONE DEACETYLASE 1 (HDAC1): A Novel therapetic target in patients with rheumatoid arthritis (RA)

***L. Göschl**[1], L. Müller[1], V. Saferding[1], S. Knapp[2], J. Backlund[3], P. Mathias[4], C. Scheinecker[1], J. Smolen[1], G. Steiner[1], W. Ellmeier[5], M. Bonelli[1];*
[1]Department of Rheumatology, MUW, Vienna, Austria, [2]Research Center for Molecular Medicine (CeMM), Austrian Academy of Sciences, Vienna, Austria, [3]Department of Medical Biochemistry and Biophysics, Medical Inflammation Research, Karolinska Institutet, Stockholm, Sweden, [4]Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland, [5]Division of Immunobiology, Institute of Immunology, Vienna, Austria.

Objectives: Certain CD4$^+$Tcells have been shown to be major drivers in RA. The expression of their key transcription factors is controlled by histone modifications which are mediated by histone deacetylases (HDAC). Indeed, pan HDAC inhibitors have been shown to be a potential therapeutic strategy. However, major side effects limit the clinical use and underline the need for more specific HDAC inhibitors. We therefore addressed the role of HDAC1 in the development of collagen-induced arthritis (CIA).
Methods: Mice with a Tcell specific deletion of HDAC1 (HDAC1cKO) were generated by using the CD4Cre/LoxP system. CIA was induced at week 8. Paraffin sections of the joints were analysed. Anti-CII antibody levels were determined by ELISA. Sera were analysed by multiplex assays. CCR6 expression in CD4$^+$Tcells was analysed by flow cytometry.

Results: Surprisingly *HDAC1cKO* mice were protected from arthritis. Anti-CII antibodies were detected in *HDAC1cKO* and *WT* mice. IL-17 was significantly decreased in the sera of *HDAC1cKO* mice when compared to *WT* mice, suggesting a role of HDAC1 in the development of Th17 cells. To see whether HDAC1 is involved in the regulation of the chemokine receptor 6 (CCR6), we stimulated $CD4^+$Tcells with IL-6. Significantly diminished levels of CCR6 were detected in $CD4^+$Tcells lacking HDAC1. This data supports the role of HDAC1 in the regulation of CCR6, which is indispensable for the migration of pathogenic Th17 cells and therefore for the development of arthritis.
Conclusion: Our data show the importance of HDAC1 as a key immune regulator in the pathogenesis of arthritis.

P.C2.10.12
Identification and analysis of anti-citrullinated protein antibodies in rheumatoid arthritis

*C. Ge[1], B. Xu[1], B. Liang[1,2], **Y. He**[1];*
[1]Section for Medical Inflammation Research, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden, [2]Department of Pathophysiology, Key Lab for Shock and Microcirculation Research of Guangdong, Southern Medical University, Guangzhou, China.

Background This project aims to investigate the anti-citrullinated protein antibodies (ACPAs) isolated from patients with rheumatoid arthritis (RA). Such antibodies have a high diagnostic value owing to their high specificity in RA. However, it is still uncertain whether these ACPAs are pathogenic or regulatory. In this project, a series of antibodies isolated from RA patients will be studied in detail both regarding specificity and function. **Method** Four antibodies (entitled as E4, F3, L2 and L4) were expressed as chimeric monoclonal antibodies with murine Fc fragment and their specificity were tested using different peptides. Functional studies of E4 and F3 were performed in mouse collagen-antibody induced arthritis (CAIA) model. The crystal structures of the antigen binding fragment (Fab) of E4 in complex with different citrullinated peptides were solved using X-ray crystallography. **Preliminary results** All four ACPAs were identically reactive with citrullinated collagen II peptides whereas they might bind to different epitopes. Most importantly, E4 antibody exhibited a strong protective effect against arthritis and bound mainly to CD11b/c+ cells. Structural studies revealed that citrulline is a critical residue and additionally interacted with the neighbouring peptide backbone. **Future plan** We have demonstrated that some ACPAs could actually be regulatory in RA in terms of our preliminary functional and structural study, the next investigation will focus on the Fab-glycosylation, the interaction between ACPAs and FcR, the targets of ACPAs and the impact of ACPAs on antigen-presenting cells and T cell in the downstream.

P.C2.10.13
Changes in CD4+ T and B cells as biomarkers of clinical response to TNF inhibitors in patients with rheumatoid arthritis

***B. Hernández-Breijo**[1,2], I. Gañán-Nieto[3], C. Sobrino[4], V. Navarro-Compán[1,5], A. Martínez-Feito[1,2], C. García-Hoz[3], J. Bachiller[4], G. Bonilla[1,5], G. Roy[3], M. Vázquez[4], A. Balsa[1,5], L. M. Villar[3], D. Pascual-Salcedo[1], E. Rodríguez-Martín[3], C. Plasencia-Rodríguez[1,5];*
[1]Immuno-Rheumatology Research Group, IdiPaz. La Paz University Hospital, Madrid, Spain, [2]Immunology Department. La Paz University Hospital, Madrid, Spain, [3]Immunology Department, Ramón y Cajal University Hospital and IRYCIS, Madrid, Spain, [4]Rheumatology Department, Ramón y Cajal University Hospital and IRYCIS, Madrid, Spain, [5]Rheumatology Department. La Paz University Hospital, Madrid, Spain.

Introduction: TNF inhibitors(TNFi) are widely used for the treatment of rheumatoid arthritis (RA). This study aims to analyse the profile of peripheral blood mononuclear cells (PBMC) after 6 months(m) of treatment with TNFi in order to find cellular biomarkers of response.
Methods: This was a prospective bi-center pilot study including 50 RA patients under TNFi therapy. PBMC were isolated from patients at baseline and 6m of treatment, and flow-cytometry analysed. Clinical activity at baseline and 6m of TNFi treatment was assessed by DAS28. Clinical remission (DAS28≤2.6) after 6m of treatment was considered as optimal response. The association between clinical remission and the percentage of change (Δ, 6m-0m) within each PBMC subset was analysed through multivariate log-regression model (odds ratio; 95% CI). All the analyses were adjusted by sex, age, concomitant-methotrexate, rheumatoid-factor and baseline-DAS28.
Results: Increased percentage of CD4+ T cells (ΔCD4+) was found after 6m of TNFi treatment in optimal responders; while suboptimal responders showed decreased percentage of this cell population (OR: 1.08; 95% CI: 1.01-1.16; p: 0.017). In addition, the percentage of B cells after 6m of TNFi treatment (ΔCD19+) decreased in optimal responders (OR: 0.7; 95% IC: 0.54-0.96; p: 0.024). This effect was essentially promoted by naïve B cells (OR: 0.7; 95% IC: 0.47-0.93; p: 0.017). The other PBMC subsets (monocytes, NK and CD8+ T cells) did not show statistical differences.
Conclusion: Our results demonstrate that CD4+ T and B cells may be useful as cellular biomarkers of response to TNFi in RA patients.
Funding: ISCIII (PI16/00474; PI16/01092)

P.C2.10.14
Autoreactive B cells against citrullinated protein antigens in rheumatoid arthritis: pathogenic roles and prospects for targeted therapy

***H. Kristyanto**[1], L. P. Lelieveldt[2], E. I. van der Voort[1], D. L. Baeten[3], H. Spits[4], K. M. Bonger[2], H. U. Scherer[1], R. E. Toes[1];*
[1]Department of Rheumatology, Leiden University Medical Centre, Leiden, Netherlands, [2]Department of Biomolecular Chemistry, Institute for Molecules and Materials, Radboud University, Nijmegen, Netherlands, [3]Department of Clinical Immunology and Rheumatology, Academic Medical Centre, Amsterdam, Netherlands, [4]Department of Cell Biology and Histology, Academic Medical Centre and AIMM Therapeutics, Amsterdam, Netherlands.

Introduction: Rheumatoid arthritis (RA) is characterized by the presence of disease-specific anti-citrullinated protein antibodies (ACPA) and remarkably responsive to the depletion of CD20+ B cells. This suggests that this B cell subset, and in particular ACPA-expressing B cells, play a central role in disease pathogenesis and could be relevant targets for therapeutic intervention.
Methods: We characterized ACPA-expressing B cells by flow cytometry in blood and synovial fluid (SF) of RA patients using streptavidin-tetramer technology and developed a dual-targeting prodrug strategy to eliminate these cells.
Results: The majority of ACPA-expressing B cells in RA blood displayed a memory phenotype (Bmem). In contrast to tetanus toxoid (TT)-specific Bmem, ACPA-expressing Bmem expressed Ki-67 and enhanced levels of CD19, CD20, CD27, HLA-DR, CD80 and CD86. In SF, ACPA-expressing B cells displayed a plasmablast/-cell phenotype and produced pro-inflammatory cytokines spontaneously. Using a cyclic citrullinated peptide (CCP) conjugated to Saporin, we could induce specific death of ACPA-expressing memory B cell clones *in-vitro*. To avoid neutralization of the antigen-drug conjugate by circulating ACPA, a carboxy-nitro-*p*-benzyl (CNBz) moiety was added to CCP-Saporin which diminished CCP recognition by ACPA. The CCP (CNBz)-Saporin prodrug could subsequently be activated in the vicinity of B cells by nitroreductase (NTR). Accordingly, NTR-treated CCP(CNBz)-Saporin induced ACPA-expressing B cell death as potently as CCP-Saporin.
Conclusion: We provide evidence that ACPA-expressing memory B cells express features by which these cells can drive inflammatory processes in RA pathogenesis. In addition, we developed an antigen-specific targeting approach for the directed elimination of this pathogenic B cell subset.

P.C2.10.15
Intranodal administration of adipose mesenchymal stem cells reduces the severity of collagen-induced experimental arthritis

*P. Mancheño-Corvo[1], **M. Lopez-Santalla**[2], R. Menta[1], O. Delarosa[1], F. Mulero[3], B. Del Rio[1], C. Ramirez[1], D. Büscher[4], J. A. Bueren[2], J. Lopez-Belmonte[5], W. Dalemans[6], M. I. Garin[2], E. Lombardo[1];*
[1]Tigenix SAU, Madrid, Spain, [2]CIEMAT/IIS Fundación Jiménez Díaz/CIBERER, Madrid, Spain, [3]Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain, [4]Grifols, Barcelona, Spain, [5]Farma-Cros, Albacete, Spain, [6]Tigenix NV, Leuven, Belgium.

INTRODUCTION: Mesenchymal stem cells (MSCs) are multipotent stromal cells that contribute to tissue regeneration, among other effects, by modulating inflammation. In recent years, novel stem cell therapy protocols for the treatment of immune-mediated disorders such as rheumatoid arthritis have been proposed. It has been demonstrated that MSCs administered systemically migrate to the draining lymph nodes suggesting that homing of MSCs to the lymphatic system plays an important role in the mechanism of action of MSCs. In this study, we proposed that direct intranodal administration of MSCs could be an alternative route of administration for MSC-based therapy.
METHODS: We analyzed the efficacy of human expanded adipose-derived mesenchymal stem cells (eASCs) to modulate established experimental collagen-induced arthritis in mice (CIA).
RESULTS: Intranodal administration of eASCs was able to modulate established experimental arthritis with a concomitant reduction in bone destruction and reduced levels of anti-chicken collagen-II IgG. We also observed an increase in the levels of regulatory T cells ($CD25^+Foxp3^+CD4^+$ cells) and Tr1 cells ($IL10^+CD4^+$), in spleen and draining lymph nodes ultimately leading to a decline in the inflammatory/regulatory balance in the tissues. CONCLUSION: Intranodal administration of eASCs may represent an alternative treatment modality for cell therapy with eASCs since it is very effective in modulating established collagen-induced arthritis.

P.C2.10.17

How do glycans affect immune cells in Rheumatoid Arthritis?

A. Molhoek[1], *L. Hafkenscheid*[2], *R. Toes*[2], *S. van Vliet*[1], *Y. van Kooyk*[1];
[1]*VUmc, Amsterdam, Netherlands,* [2]*LUMC, Leiden, Netherlands.*

The fragment antigen-binding (Fab) domain of Anti-Citrullinated Protein Antibodies (ACPAs) was recently shown to be extensively glycosylated. Glycans play a key role in controlling innate and adaptive immunity and therefore we hypothesized that the glycans on ACPA may interact with glycan binding receptors to modulate immune responses in Rheumatoid Arthritis (RA). A whole blood flow assay was used to study glycan interactions with leukocytes. Leukocytes were isolated from blood and cells were incubated for 2 hours with known immunomodulatory glycoconjugates. Glycan binding and identification of cell subsets was assessed by flow cytometry. Increased sialic acid binding to B cells, monocytes and neutrophils was observed after neuraminidase treatment to free sialic acid-binding receptors on these cells. Our preliminary data indicates that ACPA binds to B cells, NK cells and neutrophils. In addition, this assay revealed high binding of mannose specifically to B cells and monocytes. Interestingly, to date no mannose binding has been observed on peripheral B cells. In conclusion, B cells appear to be superior in their interaction capacity with a variety of glycans, including the Fab glycan on APCA. This is an important finding because B cells play a key role in the pathogenesis of RA.

P.C2.10.18

Pre-treatment leukocyte subsets as biomarkers for early identification of optimal responders to TNF inhibitors in rheumatoid arthritis

I. Nieto-Gañán[1], *B. Hernández-Breijo*[2], *C. Sobrino-Grande*[3], *C. García-Hoz Jiménez*[1], *V. Navarro-Compán*[2], *A. Martínez-Feito*[2], *J. Bachiller-Corral*[3], *G. Bonilla-Hernán*[2], *D. Pascual-Salcedo*[2], *G. Roy-Ariño*[1], *M. Vázquez-Díaz*[3], *A. Balsa*[2], *L. M. Villar-Guimerans*[1], *C. Plasencia-Rodríguez*[2], *E. Rodríguez-Martín*[1];
[1]*Immunology Department Hospital Universitario Ramón y Cajal and IRYCIS, Madrid, Spain,* [2]*Immuno-Rheumatology Research Group, IdiPaz. Hospital La Paz, Madrid, Spain,* [3]*Rheumatology Department Hospital Universitario Ramón y Cajal and IRYCIS, Madrid, Spain.*

Introduction: TNF inhibitors (TNFi) are the most common biological agents used as disease-modifying treatment in rheumatoid arthritis (RA). Although these drugs have contributed to change the natural history of RA, approximately 30-50% of patients do not respond to this therapy. Early identification of optimal responders is crucial in the clinical setting. We aimed to study if baseline percentages of different leukocyte subsets in peripheral blood(PBMCs) can contribute to identify RA patients who will respond to TNFi.
Material and methods: This was a prospective bi-center pilot study including 50 RA patients under TNFi therapy. Clinical activity was assessed at baseline and 6 months of treatment by disease activity score 28 (DAS28), considering optimal responders if they reached remission at 6 months (DAS28≤2.6). PBMCs were obtained before treatment and different leukocyte subsets were evaluated by flow cytometry in a FACSCantoII instrument. All the analyses were adjusted by sex, age, concomitant methotrexate, baseline DAS28 and rheumatoid factor through a multivariate log-regression model (odds ratio; 95% CI).
Results: Optimal responders showed higher percentage of B cells (OR=1.28; 95%; CI:1.06-1.54;p=0.011) and naive B cells (Bn; OR=1.50; 95%; CI:1.11-2.04;p=0.009) at baseline. We established cut-off values by ROC curves and analyzed the ability of both variables in predicting clinical response. Best results were obtained with Bn. Showing more than 2.55% of these cells associated with optimal response.
Conclusions: Although our data should be validated in larger cohorts, our results suggest that basal Bn percentages may contribute to identify optimal responders to TNFi in RA.

P.C2.10.19

Extensive glycosylation of anti-citrullinated protein antibodies indicates aberrant selection processes during autoreactive B-cell development

L. M. Slot[1], *R. D. Vergroesen*[1], *L. Hafkenscheid*[1], *F. S. van de Bovenkamp*[2], *M. T. Koning*[1], *B. D. van Schaik*[3], *A. H. van Kampen*[3], *H. Veelken*[1], *T. Rispens*[2], *R. E. Toes*[1], *H. U. Scherer*[1];
[1]*Leiden University Medical Center, Leiden, Netherlands,* [2]*Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands,* [3]*Academic Medical Center Amsterdam, Amsterdam, Netherlands.*

Anti-citrullinated protein antibodies (ACPA) hallmark the most disease-specific autoimmune response in Rheumatoid Arthritis (RA). ACPA-IgG are heavily *N*-glycosylated in the variable domain. The aim of the current study is to determine the frequency, origin and localisation of N-glycosylation sites in ACPA variable domains to understand the molecular basis for the remarkable glycosylation. ACPA-expressing B cells were isolated from blood of RA patients. Full-length immunoglobulin (Ig) transcripts of variable regions were obtained and analysed for the degree of somatic hypermutation (SHM) and the presence of *N*-glycosylation sites. Sequences of healthy donors (HD) served as control. 82% of ACPA-IgG, 67% of ACPA-Igκ and 47% of ACPA-Igλ contained ≥1 *N*-glycosylation sites. Despite extensive SHM, no correlation was observed between the SHM and the number of *N*-glycosylation sites. Distribution patterns and structural models indicated that *N*-glycosylation sites were predominantly located at the exterior of the molecule, away from the antigen-binding site. *N*-glycosylation sites in ACPA frequently required multiple somatic hypermutations. Computational modelling of a germinal center (CLONE algorithm), using germline counterparts of ACPA-IgG sequences, generated a pattern of *N*-glycosylation sites comparable to HD-IgG but different from ACPA-IgG. Our analyses revealed an abundance of *N*-glycosylation sites in ACPA clones. These sites frequently required multiple mutations and predominated at specific positions. This indicates that the introduction of such sites in ACPA variable regions might be a selective process that could be advantageous for the survival of ACPA-expressing B cells. Hence, variable domain *N*-glycosylation could facilitate the escape of autoreactive B cells from tolerance control mechanisms.

P.C2.10.20

Synovial tissue profiling in autoantibody positive at risk individuals reveals gene signatures associated with later development of rheumatoid arthritis

L. G. M. van Baarsen[1], *M. J. de Hair*[1], *J. F. Semmelink*[1], *I. Y. Choi*[1], *D. M. Gerlag*[1,2], *P. Tak*[1,3,4];
[1]*Amsterdam Rheumatology and immunology Center, Academic Medical Center|University of Amsterdam, Amsterdam, Netherlands,* [2]*Clinical Unit Cambridge, GlaxoSmithKline, Cambridge, United Kingdom,* [3]*Ghent University, Gent, Belgium,* [4]*University of Cambridge, Cambridge, United Kingdom.*

Objective: To study the molecular changes in synovium during the preclinical phase of rheumatoid arthritis (RA). **Methods:** We included sixty-seven individuals who were autoantibody positive and without any evidence of arthritis. All individuals underwent mini-arthroscopic synovial biopsy sampling of a knee joint at inclusion and were prospectively followed. Synovial biopsies were analyzed by transcriptomics and immunohistochemistry. **Results:** Genome-wide transcriptional profiling in a small test cohort (n=13: 6 developed arthritis) revealed many gene transcripts associated with arthritis development. Gene Set Enrichment Analysis revealed that synovial biopsies of individuals who developed RA after follow up display higher expression of genes involved in several immune response-related pathways (e.g. T cell and B cell receptor pathways, cytokine and chemokine signaling and antigen processing and presentation) compared with biopsies of individuals who did not develop RA. In contrast, lower expression was observed for genes involved in e.g. extracellular matrix receptor interaction, Wnt-mediated signal transduction and lipid metabolism. Subsequently, the expression level of 27 differentially expressed genes was validated by quantitative PCR in 61 RA-risk individuals (16 developed arthritis). Immunohistochemistry (n=54) showed an abundant expression of CXCL12 and CXCR4 already in most RA-risk individuals. Synovial biopsies from RA-risk individuals who developed arthritis were more likely to show a positive gp38 staining and lower lipid staining. **Conclusion:** This study clearly shows molecular changes appearing in synovial tissues before onset of arthritis in the absence of overt synovitis. Preclinical synovial alterations in immune response genes and lipid metabolism were associated with development of arthritis.

P.C2.11 Immune signaling and therapy in autoimmunity - Part 11

P.C2.11.01

T_H17 Cell Plasticity in Renal Autoimmune Disease

M. Zinke, *C. Kilian, B. Steglich, P. Bartsch, A. Borchers, N. Gagliani, U. Panzer, S. Huber, C. Krebs;*
Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany.

The discovery of IL-17 producing $CD4^+$ cells as a new T helper subpopulation was a huge breakthrough in the understanding of autoimmunity. T_H17 cells drive inflammatory processes and thus play a pivotal role in autoimmune diseases including crescentic glomerulonephritis (cGN). The ability to transdifferentiate from pathogenic T_H17 cells into Interferon-γ -producing T_H1 cells or into T regulatory cells is strongly limited in cGN while T_H17 cells are highly flexible in other models of autoimmune diseases like EAE and anti-CD3 duodenitis. We aim to understand the factors that contribute to T_H17 cell stability by comparative analysis of mRNA expression profiles and functional *in vivo* testing. In order to do so, we are using T_H17specific reporter mice and autoimmune mediated *in vivo* models for multiple sclerosis (EAE), cGN, intestinal inflammation (Rb^{Hi}-transfer and anti-CD3) as well as S.aureus infection. By bioinformatic comparison of transcriptomes correlated with high vs. reduced T_H17 cell plasticity we were able to identify a number of potentially interesting genes, which may play a role in this regard. Furthermore, we found high abundances of IL-27RA on renal T_H17 cells. As a result of first functional experiments, IL-27 seem to have a protective effect in cGN *in vivo*. Compared to control mice, IL-27 treated cGN mice displayed strongly reduced kidney damage. Further experiments will potentially help to elucidate essential mechanisms for T_H17 cell mediated pathogenic effects in autoimmune kidney disease. The understanding of mechanisms which may push TH17 cells into a regulatory phenotype by modulation of related pathways has a high therapeutic potential.

P.C2.11.02

Liver X receptor agonist restores impaired atherosclerotic lesion regression in rheumatoid arthritis

D. Dragoljevic[1,2]*, W. Shihata*[1,2]*, I. P. Wicks*[3,4]*, A. J. Murphy*[1,2]*;*
[1]*Baker Heart and Diabetes Institute, Melbourne, Australia,* [2]*Monash University, Melbourne, Australia,* [3]*Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia,* [4]*Royal Melbourne Hospital, Melbourne, Australia.*

The leading cause of premature mortality in patients with rheumatoid arthritis (RA) is atherosclerotic cardiovascular disease. Recently, we showed that atherosclerotic lesion regression was impaired in a murine model of RA. This was associated with cellular cholesterol efflux defects in bone marrow haematopoietic stem and progenitor cells, which promoted myelopoiesis, monocytosis and impaired atherosclerotic regression. Therefore, we hypothesised that enhanced cholesterol efflux using a Liver X Receptor (LXR) agonist, TO901317, would reduce monocytosis and improve atherosclerotic lesion regression in RA. Low-density lipoprotein receptor deficient mice were fed a western-type diet for 14wks to initiate atherogenesis, after which the mice were switched to chow to induce lesion regression. Mice undergoing regression of induced atherosclerotic lesions were divided into 3 groups; i) controls, ii) mice rendered arthritic using K/BxN serum, or iii) arthritic mice administered TO901317 (25mg/kg) daily for 2wks. TO901317 accelerated atherosclerotic lesion regression in arthritic mice, as evidenced by reduced lesion size, macrophage abundance and lipid content. Furthermore, improved lesion regression was independent to both circulating monocyte and cholesterol levels. TO901317 also reduced foam cell formation by reducing macrophage lipid loading, increasing *Abca1* and *Abcg1* expression, and increased ABCA1 protein in the atherosclerotic lesions of arthritic mice. Moreover, the severity of inflammatory arthritis was attenuated with TO901317. Taken together, we show that LXR agonism rescues impaired atherosclerotic lesion regression in a murine model of RA. This was independent of cholesterol and monocyte levels, and appeared to be due to enhanced cholesterol efflux and reduced foam cell development in atherosclerotic lesions.

P.C2.11.03

Targeting RORγt with antisense oligonucleotides impairs Th17 development in vitro

K. Schirduan, ***A. Uri****, S. Michel, F. Jaschinski;*
Secarna Pharmaceuticals GmbH&Co.KG, Munich, Germany.

RORγt is the master transcription factor of Th17 cells and constitutes an attractive therapeutic target for Th17-mediated diseases, like inflammatory bowel disease or psoriasis. Two factors are hampering the development of RORγt targeting drugs: 1) RORγt is located intracellularly and therefore inaccessible for antibodies. 2) RORγt and the ubiquitously expressed RORγ share the same DNA- and ligand-binding protein domains, impeding the development of RORγt-specific small molecule inhibitors. We here use an innovative antisense oligonucleotide based approach to selectively target RORγt but not RORγ. We designed LNA Gapmer antisense oligonucleotides (ASOs) specifically binding the RORC2 mRNA (encoding for RORγt) but not the RORC1 mRNA (encoding for RORγ) transcript variant. Treatment of a human lymphoma cell line (HDLM2) with our ASOs dose-dependently reduced RORC2 but not RORC1 mRNA expression without the need of transfection reagents. We further were able to suppress RORC2 mRNA expression in primary human $CD4^+$ T cells that were subjected to a Th17 differentiation protocol in presence of RORC2 specific ASOs. Impaired RORγt expression after ASO-treatment of human Th17 cells reduced the secretion of the Th17 effector cytokines IL-21 and IL-17. Production of IL-17 by human $CD4^+$ T cells was equally well supressed by ASOs and the small molecule inhibitors GSK805 and SR1001. Taken together, RORγt-specific ASOs provide a promising experimental therapy to treat Th17-driven diseases. Evaluation of RORγt-specific ASOs in relevant animal models will elucidate their therapeutic potential.

P.C2.11.04

Investigating clonality and function of CD4 T cells in the early phases of a murine model of experimental arthritis

S. Al-Khabouri*, R. A. Benson, C. T. Prendergast, J. M. Brewer, P. Garside;*
Institute of Infection Immunity and Inflammation, Glasgow, United Kingdom.

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that is characterised by synovial inflammation and joint erosion. $CD4^+$ T cells have been shown to play a key role in disease progression, and their ability to infiltrate joints is associated with perpetuation of local and systemic inflammatory responses. A diverse range of T cell receptor (TCR) usage has been demonstrated in RA patients, however how such diversity arises and is shaped remains unclear. Understanding this will be important for the development and application of antigen-specific therapeutic tolerance regimes. Using an experimental murine model of early arthritis, we find that the initial articular $CD4^+$ T cell response is oligoclonal in nature, with enrichment of several TCRVβ chains in the inflamed joint. The $CD4^+$ T cell response was also determined in the context of antigen and non-specific articular inflammation and the TCRVβ chain bias observed was found to be associated with an antigen driven response. Using next-generation sequencing, CDR3β sequences were analysed to determine how $CD4^+$ clonal diversity develops with disease progression and what the relative contribution of cells specific for joint antigens versus those of irrelevant specificity are to the progression of experimental arthritis. Identifying $CD4^+$ T cell clones and understanding Vβ chain usage in early RA will aid in delineating the events propagating disease. Moreover, understanding the evolution of articular $CD4^+$ T cell responses in the context of antigen specificity will inform future development of regimes to reinstate self-tolerance.

P.C2.11.05

Extracellular vesicles in rheumatoid arthritis synovial fluid contain an immunoregulatory cargo of protein and RNA

A. D. Foers[1,2]*, A. L. Garnham*[1,2]*, S. Chatfield*[1,2,3]*, L. F. Dagley*[1,2]*, A. I. Webb*[1,2]*, G. K. Smyth*[1,4]*, L. Cheng*[5]*, A. F. Hill*[5]*, I. P. Wicks*[1,2,3]*, K. C. Pang*[1,6,7]*;*
[1]*The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia,* [2]*Department of Medical Biology, University of Melbourne, Parkville, Australia,* [3]*Department of Rheumatology, Royal Melbourne Hospital, Parkville, Australia,* [4]*School of Mathematics & Statistics, University of Melbourne, Parkville, Australia,* [5]*Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Bundoora, Australia,* [6]*Department of Paediatrics, University of Melbourne, Parkville, Australia,* [7]*Murdoch Children's Research Institute, Parkville, Australia.*

Introduction In rheumatoid arthritis (RA), extracellular vesicles (EVs) are associated with both the propagation and attenuation of joint inflammation and destruction. However, specific EV content responsible for these processes is largely unknown. Investigations into identifying EV content are confounded by challenges in obtaining high-quality EV preparations from synovial fluid (SF). We recently developed a novel, size exclusion chromatography-based method of EV isolation capable of high-quality enrichments from SF (Foers *et al.*, *J Extracell Vesicles*, 2018). Here, we employed this method to investigate EV protein and RNA in SF obtained from RA and osteoarthritis patients. **Methods** Using our size exclusion chromatography-based approach, SF EVs were purified and subject to proteomics and RNAseq. Differences in protein, mRNA, and small RNA were analysed between RA and osteoarthritis patients, and between RA patients with high- and low-disease activity. **Results** Proteomics revealed RA SF EVs contain proteins associated with promoting inflammation, including tyrosine-protein kinase FER (a positive regulator of NF-κB) and components of the membrane attack complex. Further gene ontology analysis revealed an enrichment for various immunological processes, including cell-adhesion and leukocyte-mediated immunity. Additionally, we observed high-levels of the RA susceptibility gene HLA-DRB1. RNAseq revealed RA patients with high-disease activity are enriched for transcripts associated with both driving (e.g. IL-8) and attenuating (e.g. hsa-miR-27a) inflammation. **Conclusion** Our data suggest that in RA, SF EVs propagate inflammation via a pro-inflammatory protein cargo. The enrichment of HLA-DRB1 warrants further investigations into whether SF EVs present autoantigen via MHC-II. The RNA cargo suggests SF EVs traffic protein-encoding and regulatory transcripts to modulate the inflammatory phenotype of recipient cells.

P.C2.11.07

Regulation of CD20 expression by tetraspanin CD37

P. M. Hagemann[1]*, E. H. Duitmann*[1]*, F. Koch-Nolte*[2]*, F. Haag*[2]*, R. A. Manz*[3]*, Z. Orinska*[1]*;*
[1]*Research Center Borstel, Borstel, Germany,* [2]*Institute for Immunology, UKE, Hamburg, Germany,* [3]*Institute for Systemic Inflammation, Luebeck, Germany.*

B cell depletion therapy with CD20 monoclonal antibodies (mAbs) is used in treatment of lymphomas and autoimmune diseases including rheumatoid arthritis and multiple sclerosis. Among newly developed B cell depleting antibodies, CD37 is a promising candidate already in clinical trial phase II. Here we identified a crosstalk between CD20 and CD37 and indicated CD37 as a negative regulator of CD20 expression. CD37 is highly conserved in mice and humans. This makes the mouse an ideal model system to investigate CD37 induced B cell depletion and to identify potential side effects of CD37 mAb treatment. To explore the possible effects of B cell depletion therapy with CD37-specific antibodies, we generated mAbs against murine CD37 and mapped two different binding epitopes with "back to rat" mutation strategy. We show that in vitro generated plasma blasts had higher CD20 levels when co-stimulated with CD37 mAbs, compared to isotype control. CD37 mAbs alone did not activate splenic B cells, measured by CD69, CD86, MHC class II or CD25 upregulation, but lead to reduced viability (comparable with CD20 mAb control). However, CD37 mAb treatment induced an increase of proliferation in a subset of B cells, indicating activation of pro-survival pathway. In spleen, transitional and marginal zone B cells showed the highest CD37 expression levels as compared to follicular B cells. Interestingly, B cells from *Cd37-/-* mice showed strongly upregulated CD20 expression levels. A better understanding of the CD37 CD20 interaction will improve B cell directed therapies.
Funded by the DFG-RTG 1727 (Modulation of Autoimmunity).

P.C2.11.09

L-glutamine attenuates collagen-induced arthritis via inhibition of p38/NF-kB signaling in a MAPK phosphatase (MKP)-1-dependent manner

*S. Jeong, k. Kim, **S. Im**;*
Chonnam National University, Gwangju, Korea, Republic of.

L -Glutamine (Gln), a nonessential amino acid, is abundant in the plasma. Gln has been shown to have anti-inflammatory property. As a result, Gln protects animals from endotoxic shock, and attenuates many inflammatory diseases. Regarding the anti-inflammatory properties of Gln, we have shown that Gln can effectively deactivate both p38 MAPK and cytosolic phospholipase A_2 ($cPLA_2$) by rapid induction of MAPK phosphatase (MKP)-1. In this study, we explore the possibility that Gln can ameliorate collagen-induced arthritis (CIA). Oral Gln intake (0.5 g/kg/day) attenuated not only severity of arthritis based on clinical scores, hind paw thickness, and radiographic and pathologic findings, but also local and systemic levels of proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, and leukotriene B4. Oral Gln inhibited p38 phosphorylation and NF-κB activation in the inflammed tissues. Gln administration resulted in early and enhanced MKP-1 induction. Importantly, MKP-1 small interfering RNA (siRNA), but not control siRNA, significantly abrogated the Gln-mediated 1) induction of MKP-1; 2) attenuation of arthritis; 3) inhibition of p38 phosphorylation, NF-κB activation and tissue levels of proinflammatory cytokines. These data indicated that Gln ameliorated CIA via inhibition of p38/NF-κB signaling in a MKP-1-dependent manner.

P.C2.11.10

Insulin modulation of TLR4 expression in murine macrophages: possible involvement of PI3K/Akt and ERK1/2 signalling pathway

***S. Pal**, S. Maitra;*
Visva-Bharati University, Bolpur, India.

Toll-like receptor (TLR) mediated diet-induced obesity or insulin resistance is involved in the pathogenesis of type 2 diabetes. In obesity, macrophage accumulation in insulin target tissues (visceral adipose tissues) and TLR4 dependent up-regulation of cytokines promotes chronic inflammation, which in its turn leads to diabetic complications including nephropathy, atherosclerosis and retinopathy. Conversely, reducing glucose levels with insulin therapy is associated with decreased inflammation, mortality and incidence of sepsis in critically ill patients. However, importance of insulin signalling in macrophage function and polarization is not well characterized and molecular mechanisms underlying insulin modulation of TLR4 induction in murine macrophages is yet to be determined. In this study participation of insulin-mediated PI3K/Akt and ERK1/2 signalling during high glucose (HG) and/or lipopolysaccharide (LPS)-induced TLR4 expression in murine macrophages has been investigated. Present results show that while expression of insulin receptor (IR) remains unchanged, insulin alone could attenuate HG and/or LPS -induced TLR4 expression in duration-dependent manner, at both mRNA as well as protein level. Further, insulin either alone or in presence of HG and LPS could up-regulate PI3K/Akt and ERK1/2 phosphorylation (activation) *in vitro*. More interestingly, in PI3K as well as ERK1/2 inhibited cells (using wortmannin and U0126 respectively), insulin failed to reverse HG or LPS action on TLR4 induction completely. Collectively, participation of PI3K/Akt and ERK1/2 signalling cascades has pivotal influence in insulin modulation of TLR4 expression induced by glucose or LPS in murine macrophages. This area of research is beneficial for further understanding of the interplay between insulin signalling and host immune system in metabolic diseases.

P.C2.11.11

Are controls in trans the production of medullary thymic epithelial cells expressing Ly6C/Ly6G

***M. Matsumoto**, J. Morimoto, M. Matsumoto, H. Nishijima;*
Institute for Enzyme Research, Tokushima, Japan.

Medullary thymic epithelial cells (mTECs), which express a wide range of tissue-restricted self-antigens (TRAs), contribute to the establishment of self-tolerance by eliminating autoreactive T-cells and/or inducing regulatory T-cells. Aire controls a diverse set of TRAs within Aire-expressing cells by employing various transcriptional pathways. As Aire has a profound effect on transcriptomes of mTECs including TRAs not only at the single-cell but also the population level, we suspected that Aire ($Aire^+$ mTECs) might control the cellular composition of the thymic microenvironment. Here, we confirmed that this is indeed the case by identifying a novel mTEC subset expressing Ly6 family protein whose production was defective in Aire-deficient thymi. Re-aggregated thymic organ culture experiments demonstrated that Aire did not induce the expression of Ly6C/Ly6G molecules from mTECs as Aire-dependent TRAs in a cell-intrinsic manner. Instead, $Aire^+$ mTECs functioned in trans to maintain $Ly6C/Ly6G^+$ mTECs. Thus, Aire not only controls TRA expression transcriptionally within the cell, but also controls the overall composition of mTECs in a cell-extrinsic manner, thereby regulating the transcriptome from mTECs on a global scale.

P.C2.11.12

33°C provides T regulatory cells stability

***K. Piekarska**[1], N. Filipowicz[2], A. Piotrowski[2], M. Gucwa[2], J. Sakowska[3], K. Vogt[4], B. Sawitzki[4], J. Siebiert[5], P. Trzonkowski[3], N. Marek-Trzonkowska[1];*
[1]Laboratory of Immunoregulation and Cellular Therapies, Department of Family Medicine, Medical University of Gdansk, Gdańsk, Poland, [2]Department of Biology and Pharmaceutical Botany, Medical University of Gdańsk, Gdańsk, Poland, [3]Department of Clinical Immunology and Transplantology, Medical University of Gdansk, Gdańsk, Poland, [4]Institute for Medical Immunology, Charité – Universitätsmedizin Berlin, Berlin, Germany, [5]Department of Family Medicine, Medical University of Gdańsk, Gdańsk, Poland.

Introduction – Regulatory T cells (Tregs) play crucial role in self-tolerance and tissue homeostasis. Nowadays, regulatory T-cell therapy is considered safe and effective, for instance in type 1 diabetes treatment. However, Tregs lose their characteristic phenotype and suppressive potential during expansion ex vivo.
Methods – Tregs were freshly isolated from buffy coats obtained from 13 volunteer blood donors. After sorting Tregs were equally divided into 2 separate cultures and expanded at 37°C and 33°C. Each 7th and 14th day of the expansion Tregs were checked for phenotype and function with flow cytometry. In addition, DNA methylation of the Treg-Specific Demethylated Region (TSDR) and RNA-seq were performed for Tregs cultured in both studied culture temperatures, as well as for CD4+ effector T cells (Teffs) derived from the same donors.
Results – 33°C induces robust proliferation of Tregs (4.5-fold higher cell counts as compared with Tregs expanded at 37°C), enhances expression of FoxP3, CD25, Helios, CD39, and CTLA-4 in Tregs and keeps Treg phenotype stable during the culture. Tregs expanded at 33°C were characterized by significantly higher frequency of cells with demethylated TSDR and presented remarkably anti-inflammatory phenotype after culture *in vitro*, as compared with cells at 37°C.
Conclusion – Tregs expanded at 33°C have stronger immunosuppressive potential. Mild hypothermia can preserve Treg phenotype, function and accelerate their proliferation, responding to unanswered question- how to preserve Treg stability in vitro.

P.C2.11.13

Anti-Serotonin antibodies in Fibromyalgia patients

***L. Gimeno**[1], A. Mrowiec[1], P. Martinez[1], G. Salgado-Cecilia[1], E. Novoa- Bolivar[1], J. M. Bolarin[1], O. Montes-Ares[1], A. Bermudez-Torrente[2], A. Martinez-Leon[3], A. Minguela-Puras[1], I. Lozano- Olmos[4], R. Klein[5], A. M. Garcia-Alonso[1];*
[1]Immunology Service-University Clinical Hospital Virgen de la Arrixaca-IMIB, El Palmar, Spain, [2]Rheumatology Service-University Clinical Hospital Virgen de la Arrixaca-IMIB, El Palmar, Spain, [3]Medical Family Health San Andres-Servicio Murciano de Salud, Murcia, Spain, [4]Psychiatry Service-University Clinical Hospital Virgen de la Arrixaca-IMIB, El Palmar, Spain, [5]Laboratory for Immunopathology and HLA, Eberhard Karls University Tübingen, Tübingen, Germany.

Fibromyalgia (FM) is an idiopathic disorder characterized by widespread nonarticular musculoskeletal pain accompanied by fatigue, sleep and memory disorders and mainly affecting women. The etiopathogenesis of FM is still unknown but evidence suggests an inflammatory and autoimmune origin characterized by an autoantibody pattern. Presence of antibodies to serotonin (5-hydroxytrypyamine) has been found in about 70% of German FM patients and appears to be characteristic for this disease. Our aim was to evaluate the presence of antibodies to serotonin in serum of Spanish type I FM patients. The study included 156 clinically defined FM patients and 153 healthy controls. Sera from patients and control subjects were tested by ELISA against Serotonin. Anti-serotonine antibodies were found in 65 % of the patients suffering from FM. Statistically significant difference was found with respect to healthy controls that presented 14% ($Pc<0.05$). Our data corroborate German results, presence of antibodies to serotonine in FM patients.
The diagnostic relevance of these antibodies is supported by the absence of anti-serotonin antibodies in other rheumatic disorders. The association of anti-serotonine antibodies with psychiatric disorders in FM is thought to represent at least partially a dysregulation of the serotonergic neurotransmitter system in the central nervous system. Although these immunological results are still beyond our understanding, they could indicate an autoimmune component of the disease regardless of inflammatory response or alterations of the neuroendrocrine system. Broader studies will be necessary in the future.

P.C2.11.14

Immune gene signature in kidneys of patients diagnosed with antineutrophil cytoplasmic antibody-associated vasculitis

*E. García Moreno[1], R. De la Varga Martínez[2], C. Rodriguez Hernández[1], **A. Sampalo Laínz**[1];*
[1]Immunology Department, Puerta del Mar Hospital, Cádiz, Spain, [2]Immunology Department, Virgen del Rocio Hospital, Sevilla, Spain.

Introduction: Antineutrophil cytoplasmic antibody-associated vasculitis (AAV) are systemic necrotizing small-vessel vasculitis characterized by glomerulonephritis, granulomatous inflammation and pauci-immune necrotizing small-vessel vasculitis. Knowledge regarding the mechanisms involved in AAV has increased. Nevertheless, relapsing renal disease is still a major issue. Our objective was to study the immune-related gene expression in kidneys from patients diagnosed with AAV.

Materials and Method: The .cel data files were uploaded to Gene Expression Omnibus Database (series accession number GSE104948 and GSE104954). We select 46 glomerular and tubular samples from kidney biopsies from 23 patients diagnosed with AAV and, as a control, from 21 transplant living donors. Differential gene expression analysis was performed by using the R/Bioconductor package, *limma.* For biological function analysis, DAVID Bioinformatics Resources were used.
Results: A total of 1783 differentially expressed genes in patients were analysed. The 412 genes with the same pattern of expression (up or down regulated) in both the glomerular and tubular tissues were selected. The functional analysis of the 311 up regulated genes revealed that there was a statistically significant enrichment in pathways involved in chemokine signalling (25 genes), Cytokine-cytokine receptor interaction (25), leukocyte transendothelial migration (14), complement cascade (10), phagocytosis(11), and Natural killer cell mediated cytotoxicity (14).
Conclusions: We have found an immune and inflammatory gene signature in kidneys from AVV patients. Most of up-regulated genes were related to cytokines, chemokines and other factors involved in the activation, growth and movement of immune cells. Therefore, they could be potential targets in renal AAV.

P.C2.11.15
Human DC-SIGN and CD23 do not bind directly to sialylated human IgG

R. Temming[1], *G. Dekkers*[1], *S. van de Bovenkamp*[1], *A. Bentlage*[1], *T. Rispens*[1], *R. Plomp*[2], *M. Wuhrer*[2], *G. Vidarsson*[1];
[1]*Sanquin, Amsterdam, Netherlands,* [2]*LUMC, Leiden, Netherlands.*

The precise mechanism underlying the anti-inflammatory effect of intravenous immunoglobulin (IVIg) therapy remains to be fully elucidated. The potential role of sialylated IgG within IVIg has drawn considerable interest, because this fraction has been found to be more therapeutically active in some mouse models. It has been proposed that upon Fc-sialylation, IgG undergoes a conformational change resulting in closure for conventional FcγRs and simultaneous opening for SIGNR-1 (homologue of human DC-SIGN) and CD23. These latter receptors have been demonstrated to be responsible for the IVIg effect of sialylated human IVIg in mouse models.
However, the precise mechanisms of the interaction between human IgG and DC-SIGN/CD23 are unknown and await confirmation, particularly in the human setting.
This study dissected the binding of human IgG1-glycoforms to DC-SIGN/CD23 in their native configuration. While cells expressing these receptors bound to their native ligands, no binding was observed by FACS regardless of sialylation. More sensitive cellular surface plasmon resonance imaging, to study avidity effects, also did not show binding of any antigen-complexed IgG-glycoform.
In conclusion, these findings indicate that no direct binding between human IgG and DC-SIGN/CD23, regardless of the glycosylation status. This suggests that previously obtained mouse data does not apply for humans.

P.C2.11.16
Immunomodulatory drugs humanization do not avoid immunogenicity

L. Díez Alonso[1,2], *E. Gómez Massa*[1,2], *L. Naranjo Rondán*[1,2], *O. Cabrera Marante*[1,2], *A. Serrano Hernández*[1,2];
[1]*Hospital Universitario 12 de Octubre, Madrid, Spain,* [2]*Instituto de Investigación, Hospital Universitario 12 de Octubre, Madrid, Spain.*

Biological immunomodulatory drugs blocking TNF-α mediated responses are largely used as a treatment for immuno-mediated diseases. Despite its beneficial effects, the treatment may be complicated by the development of unwanted immunogenicity. The development of anti-drug antibodies is associated with important clinical impact in terms of safety and efficacy. The aim of our study was to asses if the origin of the biological drug (murine, humanized or human) affects antibody development.
Blood samples from 484 patients treated with these immunomodulatory drugs at 12 Octubre Hospital were collected during 29 moths between 2016 and 2018. All serum samples were evaluated for drug levels and those that resulted in undetectable drug levels were evaluated for the presence of non-isotype specific anti-drug antibodies using a commercially available bridging format ELISA kit (Promonitor®, Progenika Biopharma S.A., Spain), according to the manufacturer's instructions.
Twenty three out of 257 patients with murine monoclonal antibody infliximab (8.95 %), 21 out of 207 (10.14%) patients with human monoclonal antibody adalimumab and any patient with human recombinant protein etanercept developed anti-drug antibodies. The anti-drug antibodies presence didn't seem to be significantly different between infliximab (murine) and adalimumab (human). Anti-drug antibodies triggers negatively influences to the treatment response so it is important to determine the factors that can promote its development. In this study, we demonstrate that the origin of biological immunomodulatory drugs do not affect the later anti-drug antibody production. Owing to these monoclonal antibodies are produced in the same cell line, post-translational modifications could be the shared immunogenic cause.

P.C3.01 Bone Marrow Transplantation

P.C3.01.01
The glucocorticoid receptor in recipient cells keeps cytokine secretion in acute graft-versus-host disease at bay

T. K. Baake;
Institute for Cellular and Molecular Immunology, Georg-August-University, University Medical Center, Göttingen, Germany.

Introduction: Acute Graft-versus-host disease (aGvHD) is a life-threatening complication of hematopoietic stem cell transplantation (HSCT) and considered as the main risk factor for transplant-related morbidity and mortality. First-Line therapy of GvHD is usually accomplished by glucocorticoid (GC) application but the mechanisms and target cells of this treatment regimen are not yet fully understood. Objectives: Here we analyzed the role of the glucocorticoid receptor (GR) in recipient myeloid cells and determined how its deletion in mice influences mortality, clinical symptoms, intestinal tissue damage as well as local and systemic cytokine production after allogeneic HSCT. Materials & methods: A total MHC-mismatched model of allogeneic HSCT was used to induce aGvHD in GR^{lysM} mice lacking the GR in myeloid cells. Bone marrow and purified T cells were isolated from wildtype C57BL/6 mice and transferred into irradiated GR^{flox} and GR^{lysM} BALB/c mice. The disease course was followed over a period of 6 weeks, histological and immunological analyses were performed on day 4, 6 and 8 post-transplant. Results: Deletion of the GR in recipient myeloid cells strongly exacerbates aGvHD, an effect which is unrelated to local GC effects in GvHD target organs but rather caused by uncontrolled systemic cytokine release. Conclusion: The regulation of recipient myeloid cells by GC is essential to prevent a lethal cytokine storms after allogeneic HSCT. Selectively targeting this cell type might therefore offer a new strategy to treat aGvHD patients.

P.C3.01.02
Outcome of mesenchymal stromal cell treatment in chronic GvHD predicted by thymic function: A phase II clinical study

E. Boberg[1], *L. von Bahr*[1], *N. Heldring*[2], *G. Afram*[3], *E. Alici*[1], *E. Iacobaeus*[1], *K. Garming Legert*[4], *P. Petzelbauer*[5], *P. Ljungman*[6], *R. Sugars*[4], *N. Kadri*[1], *K. Le Blanc*[1];
[1]*Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden,* [2]*Center for Hematology and Regenerative Medicine, Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden,* [3]*Department of Cellular Therapy and Allogeneic Stem Cell Transplantation, Karolinska University Hospital, Stockholm, Sweden,* [4]*Department of Dental Medicine, Karolinska Institutet, Stockholm, Sweden,* [5]*Skin & Endothelial research division SERD, Department of Dermatology, Medical University of Vienna, Vienna, Austria,* [6]*Division of Hematology, Department of Medicine Huddinge, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.*

Introduction: Treatment options are limited for chronic graft-versus-host-disease (cGvHD) refractory to steroid treatment. Infusion of mesenchymal stromal cells (MSCs) is a promising alternative but the mechanism of action has not been established.
Methods: 11 patients with severe steroid refractory cGvHD were treated with repeated infusions of allogeneic bone marrow MSCs. Clinical response was evaluated using the National Institutes of Health (NIH) criteria. Immunophenotyping and cytokine analysis was conducted on blood samples taken before, and at several time points shortly after each infusion. Skin biopsies were taken before and after treatment.
Results: Nine patients completed the treatment protocol and were evaluated for response. Six patients responded to MSC therapy according to NIH criteria. The responders had significantly higher proportions of naive Th-cells (CD3+CD4+CD45RA+CD27+) and activated naïve B cells (CD19+IgD+CD38low). The proportion of Ki67+ naïve Th-cells was similar in both responders and non-responders, while the percentage of recent thymic emigrants among naïve CD4+ T-cells were higher in responders, suggesting a better thymic function. Several miRNAs increased one hour after MSC infusion in all patients, followed by transient increases in absolute numbers of naïve and regulatory T- and B-cells after 7 days in responders. Long term reductions were seen in the cytokines CXCL2 and CCL2 in responders, while the levels of CXCL9, CXCL10, CXCL12, TNFα and IL-6 increased in non-responders. Skin biopsies showed resolution of epidermal pathology.
Conclusion: Our data highlights the importance of the recipient immune phenotype for response to MSC treatment of cGvHD and suggests a link to thymic function.

P.C3.01.03
In silico analysis of alloantigen landscape in HLA-matched transplantation

N. A. Bykova*, D. B. Malko, G. A. Efimov;*
National Research Center for Hematology, Moscow, Russian Federation.

Minor histocompatibility antigens (MiHAs) are alloantigens in the context of HLA-matched allogeneic hematopoietic stem cells transplantation (allo-HSCT). Due to the crucial role they play in graft versus leukemia effect MiHAs were suggested as possible targets for immunotherapy. However, the restricted number of clinically relevant MiHAs known so far limits their applicability. To make anti-MiHA therapy broadly usable systematic discovery of novel MiHAs is needed. In the current study we performed in silico analysis of the genomic data to describe basic features of MiHA landscape. For this, pairs of samples were arbitrary selected from 1000 Genome project, as donor and recipient. Unique peptides found in the recipient genome that were predicted to bind MHC molecules with high affinity were considered as potential MiHAs.

We further compare the results with known and mass spectrometry (MS) predicted MiHAs. The analysis demonstrated that at the moment our knowledge about MiHAs is largely inconsistent: MS approach to predict new MiHAs poorly reproduces known MiHAs, probably missing significant amount of potentially relevant MiHAs; the ratio of co-dominant/dominant MiHAs is inconsistent between in silico predicted and MS predicted or known MiHAs; MS predicted peptides are biased towards high allele frequency of immunogenic allele, which is suboptimal for usage in immunotherapy. Lower estimations of in silico approach suggest that there are at least several dozen strong MHC-binding SNP-associated peptides in the allele frequency region optimal for off-the-shelf therapeutics, that are still not experimentally tested for immunogenicity. Funding was provided by Russian Science Foundation grant 17-15-01512.

P.C3.01.04

Discovery of HLA class I-restricted minor histocompatibility antigens by a new approach for whole genome association scanning

***K. J. Fuchs**, A. E. Adriaans, M. W. Honders, E. D. van der Meijden, M. J. Pont, R. Monajemi, S. M. Kielbasa, P. A. ,t Hoen, C. A. van Bergen, J. Falkenburg, M. Griffioen;*
Leiden University Medical Center, Leiden, Netherlands.

Minor histocompatibility antigens are polymorphic peptides presented on the cell surface by HLA molecules. After allogeneic stem cell transplantation (SCT) as treatment for hematological malignancies, genetic differences in single nucleotide polymorphisms (SNPs) between patient and donor result in presentation of minor histocompatibility antigens that are recognized by donor T-cells. These T-cells can induce the favourable Graft-versus-Leukemia effect, but also mediate undesired Graft-versus-Host Disease. In our laboratory, whole genome association scanning has been developed to identify minor histocompatibility antigens. In this method, donor T-cells isolated from patients after allogeneic SCT are tested for recognition of a panel of 80 B-lymphoblastoid cell lines, and SNPs that strongly associate with T-cell recognition are subsequently validated to encode the antigens. The number of SNPs that were measured for this panel, however, was limited to one million. Utilizing a new panel of 191 B-cell lines, which are sequenced in the 1000 Genome Project, we now optimized the approach and increased SNP coverage to 12 million. Furthermore, while our previous panel was restricted to few HLAs, the new panel includes six common HLAs. This approach for whole genome association scanning has been successfully applied and various new minor histocompatibility antigens have been found. The aim is to use the optimized method to identify the dominant repertoire of HLA class I-restricted minor histocompatibility antigens, since knowledge about mismatched antigens may allow for a personalized treatment after transplantation as well as a more directed donor selection thereby contributing to a better outcome of patients treated with allogeneic SCT.

P.C3.01.05

The impact of stem cell graft γδT cells on clinical outcome after allogeneic hematopoietic stem cell transplantation

***A. Gaballa**[1], A. Stikvoort[1], B. Önfelt[2,1], J. Mattsson[1], M. Sundin[1], E. Watz[1], M. Uhlin[1,2];*
[1]Karolinska Institutet, Stockholm, Sweden, [2]Royal Institute of Technology, Stockholm, Sweden.

The impact of donor graft composition of T cells on the clinical outcome following allogeneic hematopoietic stem cell transplantation (HSCT) has been thoroughly investigated. However, most of studies have focused on the role of alpha beta ($\alpha\beta^+$) T cells and less attention has been given to the impact of gamma/delta ($\gamma\delta^+$) T cells. Whether $\gamma\delta^+$ T cells are beneficial or not for patient outcome, especially with regards to Graft versus Host Disease (GvHD) is still questionable. In this study, we analysed samples from 105 donor grafts, and the frequencies of $\gamma\delta^+$ T cell subsets were correlated with the clinical outcome. We show for the first time that grafts containing higher proportions of CD8+γδ T cells were associated with increased cumulative incidence of aGvHD II-III. We also found that graft frequency of CD27+γδ T cells was inversely correlated with CMV reactivation and relapse. Our findings illustrate the importance of better understanding of the role played by distinct γδ T cell subsets in allogeneic HSCT.

P.C3.01.07

The CD73/A2A signalling axis impacts graft-versus-host disease in a humanised mouse model

***N. J. Geraghty**, S. R. Adhikary, D. Watson, R. Sluyter;*
University of Wollongong, Wollongong, Australia.

Introduction: Graft-versus-host disease (GVHD) is a major cause of mortality following allogeneic hematopoietic stem cell transplantation. GVHD occurs when T cells in the graft cause inflammatory damage to host tissues. Ecto-5'-nucleotidase (CD73) generates extracellular adenosine, which activates the adenosine 2A (A2A) receptor to limit T cell responses. In allogeneic mouse models of GVHD, CD73 or A2A blockade worsens, whilst A2A activation limits disease. The current study examined the role of the CD73/A2A signalling axis in a humanised mouse model of GVHD. **Material and Methods:** NOD-SCID-IL2γ^{null} (NSG) mice, injected i.p. with $10x10^6$ human (h) peripheral blood mononuclear cells, were subsequently injected i.p. with αβ-methyleneADP (CD73 antagonist) or CGS21680 (A2A agonist) for 6 or 14 days, respectively. GVHD development was assessed by weight loss, clinical parameters, survival and histology. Immune cell markers and cytokines were analysed by flow cytometry. **Results:** Neither CD73 blockade or A2A activation impacted initial human leukocyte engraftment or survival. CD73 blockade worsened GVHD as evidenced by increased weight loss, liver histology and serum hIL-2. Contrary to expectations, A2A activation increased weight loss, decreased human regulatory T cells and increased serum hIL-6. A2A activation however decreased serum hTNF-α and hIL-2, but did not alter histology of target tissues. **Conclusion:** CD73 blockade or A2A activation reduces the health of mice in this humanised model of GVHD. The CD73 blockade effects may be explained by accumulation of proinflammatory ATP rather than reduced adenosine production. The effects of A2A activation may reflect reduced metabolism or food intake rather than GVHD per se.

P.C3.01.08

The fast NKG2C+ NK "memory" expansion in CMV reactivation patients post allogeneic Stem Cell Transplantation

***N. Hassan**[1,2], J. Zuo[1], S. Eldershaw[1], J. Nunnick[3], P. Moss[1,3];*
[1]Institute of Immunology and Immunotherapy, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom, [2]Institute of Cancer and Genomic Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom, [3]Birmingham Health Partners, Centre for Clinical Haematology, Queen Elizabeth Hospital, Birmingham, United Kingdom.

Introduction: Human cytomegalovirus (HCMV) reactivation in allogeneic haematopoietic stem cell transplantation (HSCT), regularly leads to HCMV disease and can cause serious morbidity and mortality. The relation between CMV-reactivation and leukaemia recurrence in patients post HCST is highly controversial and there is increasing evidence to show that CMV-reactivation is associated with the decrease rate of Leukaemia relapse post HSCT.
Methods: The phenotype of NK cell in the peripheral blood mononuclear cells (PBMCs) in HSCT patients with HCMV-reactivation was studied using flow cytometry. The HSCT patients without HCMV-reactivation and healthy donors PBMC were used as a control. The markers analysed for NK phenotypes include CD56, CD16, CD94/NKG2C, CD94/NKG2A, KIRs, CD57, KLRG1 and PD-1.
Results: NK cell surface expression markers were compared between HSCT patients with HCMV-reactivation and without HCMV-reactivation. The NK cells from both patient groups have similar composition of $CD56^{dim}/CD16^{bright}$ and $CD56^{bright}/CD16^{neg}$. Strikingly, the percentage of $CD56^{dim}16^{bright}$NKG2C+ population in HCMV-reactivation patients accumulated overtime when compared with no reactivation. Before HSCT, there are 6.4±3.5% (Mean+SEM) NKG2C+ NK cells in HCMV-reactivation group comparing with 2.9±1.4% NKG2C+ NK cells in non-reactivation group. 10 months later, there are significantly higher NKG2C+ NK cells in HCMV reactivation group (35.8±10.4% versus 8.8±2.9%).
Conclusions: This study has shown the fast accumulation of $CD56^{dim}16^{bright}$NKG2C+ NK population in HSCT patients with HCMV-reactivation. We hypothesise that NK population will contribute to the reduction of Leukemia relapse in HSCT patients with HCMV-reactivation. This study will help to guide the future management of HCMV-reactivation and develop strategies to reduce disease relapse.

P.C3.01.09

Exploring immunological mechanisms of human graft-versus-host-disease after hematopoietic stem cell transplantation

***E. Latis**[1,2], C. Leloup[1], D. Michonneau[3], A. Garcia[3], E. Bianchi[1], G. Socié[3], L. Rogge[1];*
[1]Institut Pasteur, Immunoregulation Unit, Department of Immunology, Paris, France, [2]Université Paris Diderot, Paris, France, [3]Service Hematologie Greffe, Hôpital St. Louis, Paris, France.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment for many hematologic malignancies. However, its success is hindered by graft-versus-host disease (GVHD), a life-threatening complication deriving from alloreactive donor T-cells attacking recipient (host) tissues. Despite the advances in the field of HSCT and GVHD prophylaxis, disease processes in humans remain poorly understood, and the lack of biomarkers for the early diagnosis and prognosis contributes to the high mortality of the disease. In this study we investigated the mechanisms involved in immune reconstitution early after transplantation and in acute GVHD onset. For two independent cohorts of related HLA-identical donor-recipient couples, blood was collected either at GVHD onset, before any treatment, or at day 90 post-HSCT for recipients not developing GVHD. We have performed molecular profiling of cell populations important for GVHD development as well as immuno-phenotyping using spectral flow cytometry. Molecular profiling showed that donor T-cells react to the environment of the host by acquiring an activated phenotype, upregulating genes associated with T-cell activation, co-stimulation, migration and effector functions. Cellular profiling showed an incomplete reconstitution of the T-cell compartment in recipients early after transplantation, and a depletion of naive T-cells associated with an increase of cells with an effector-memory phenotype after HSCT. Finally, we noted an increase of cells with a T memory stem cell-like (TSCM-like) phenotype at GVHD onset. These cells may represent a cellular reservoir for GVHD maintaining the production of alloreactive T cells in the presence of host persistent antigens.

P.C3.01.10

Absence of host macrophages exacerbates acute GVHD

D. Le[1], *M. Ranecky*[1], *A. Jordán Garrote*[1], *K. Ottmüller*[1], *H. Shaikh*[1], *M. Qureischi*[1], *L. Scheller*[1], *T. Steinfatt*[1], *A. Brandl*[1], *Z. Mokhtari*[1], *J. Delgado Tascón*[1], *J. Hartweg*[1], *A. Mottok*[2], *H. Einsele*[1], *E. Vafadarnejad*[3], *A. Saliba*[3], *M. B. Lutz*[4], *A. Beilhack*[1];
[1]*Department of Medicine II, Wuerzburg, Germany,* [2]*Institute of Pathology, Wuerzburg, Germany,* [3]*Helmholtz Institute for RNA-based Infection Research (HIRI), Wuerzburg, Germany,* [4]*Institute for Virology and Immunobiology, Wuerzburg, Germany.*

Allogeneic hematopoietic cell transplantation (allo-HCT) can lead to the severe complication of intestinal acute graft-versus-host disease (aGVHD). Host tissue resident antigen-presenting cells are considered essential in aGVHD initiation. However, in recent years it has become clear that hematopoietic host APCs are not absolutely required to initiate acute GvHD and that non-hematopoietic host APCs are sufficient to effectively activate alloreactive T cells to trigger acute GvHD. Therefore, the role of hematopoietic APCs in GvHD has not yet been clearly def.
Here we report that depletion of host APCs, which highly express CD11c, leads to exacerbation of GvHD as seen from reduced survival and increased clinical score after allo-HCT. Using reporter mice for dendritic cells (Zbtb46-GFP), novel macrophage marker panels for flow cytometry (MHC II, CD11c, F4/80, CD64, CD26) and single cell sequencing we show for the first time that the host intestinal CD11c expressing myeloid cells, which survived after irradiation, are macrophages but not dendritic cells. Moreover, our results indicate, that host macrophages can reduce intestinal aGVHD via PD-L1.
Our data indicate a protective role of host macrophages in aGVHD and suggest that targeting intestinal host macrophages may represent a novel therapeutic strategy for aGvHD.

P.C3.01.11

Predictive Surface Biomarkers for Murine Acute Graft-versus-Host Disease

M. Qureischi[1], *C. Bäuerlein*[1], *C. Brede*[1], *A. Jordan Garrotte*[1], *S. Riedel*[1], *M. Chopra*[1], *A. Mottok*[1], *S. Thusek*[1], *M. Ritz*[1], *K. Mattenheimer*[1], *C. Graf*[1], *H. Einsele*[1], *R. Negrin*[2], *P. Schlegel*[3], *A. Beilhack*[1];
[1]*Centre for Experimental Molecular Medicine, Würzburg, Germany,* [2]*Division of Blood and Marrow Transplantation, Stanford University, Germany,* [3]*Department of Pediatrics, Würzburg, Germany.*

Acute graft-versus-host disease (aGvHD) is a severe inflammatory complication of hematopoietic cell transplantation. aGVHD is mediated by donor T cells attacking the gastrointestinal tract, liver, and skin. Efficient strategies to improve aGvHD-related morbidity and mortality will rely on more precise methods to predict aGvHD and abrogate disease manifestation. Here, we asked whether the combination of surface receptors, particularly chemokine receptors and adhesion molecules, would identify alloreactive donor T cells before the onset of acute GVHD. To this end we employed multiparameter flow cytometry in two independent murine allo-HCT models to address whether defined markers alone or in combination could predict the onset of GvHD. C57Bl/6 (H-2b, Thy1.1+) or B10.D2 (H-2d, Thy1.1+) T cells plus bone marrow cells were transplanted in conditioned (8Gy) miHAg mismatched BALB/B (H-2b, Thy1.2+) and syngeneic C57Bl/6 (9Gy) or BALB/c (H-2d, Thy1.1+) recipients. To identify suitable predictive markers, we compared the expression pattern of allo-HCT recipients to syngeneic HCT recipients and untreated controls. Notably, the chemokine receptor expression (CCR2, CCR4, CCR5, CCR6, CCR7, CCR9) did not differ in the PB of allogenic vs. syngenic transplanted mice. However, we found an upregulation of α4β7 integrin, and E- and P-selectin ligand on allogeneic PB T-cells early after allo-HCT. The combination of these homing markers with the activation markers CD25 and CD69 at later time points and low expression levels of L-selectin allowed to define alloreactive donor T-cells. Based on these data, we propose a potential predictive blood test, which could allow a timely therapeutic intervention before clinical aGvHD manifestation.

P.C3.01.12

Serine protease activated Protein C alters T cell plasticity in GvHD by inducing anergy and metabolic quiescence in Teffector cells

S. Ranjan[1], *D. Gupta*[1], *A. Goihl*[2], *B. Schraven*[2], *B. Isermann*[1];
[1]*Institute of Clinical Chemistry and Pathobiochemistry, Magdeburg, Germany,* [2]*Institute for Molecular and Clinical Immunology, Magdeburg, Germany.*

Introduction: The serine protease activated protein C (aPC) is an anticoagulant protease. The role of aPC in controlling innate immunity through both its anticoagulant and signaling properties is well recognized. We have recently demonstrated a novel function of aPC and its receptors (protease activated receptors) in adaptive immunity and shown that aPC ameliorates GvHD. Here we test the hypothesis that aPC regulates T cell plasticity in GvHD. Material and Methods: In vitro proliferation assays were done by mixed lymphocyte culture of human and mice Teff cells. Teff cell anergy markers were measured by FACS and OCR and ECAR were measured in Teff cells by Seahorse. GvHD was induced in BALB/c mice by transplanting $2x10^6$ T cells with $5x10^6$ bone marrow cells from C57BL/6 mice. Results: *In vitro* we observed induction of iTregs (CD4+CD25+Foxp3) from Teff (CD4+CD25$^-$ Foxp3^{lo}) cells. This change in T cell plasticity is associated with induction of anergy (induced CD73 and A2A receptor expression, reduced proliferation and IL2 secretion) and metabolic quiescence (reduced glycolysis and mitochondrial respiration) in Teff cells after 24h of stimulation. *In vitro* aPC also reduced phosphorylation of Akt, mTORC1 and p70S6 kinase in human Teff cells. *In vivo* aPC improves GvHD in mice by inducing anergy and altering T cell plasticity. This effect is associated with improved survival, lower clinical scores, and lesser tissue damage and apoptosis in the gut. Conclusions: This data demonstrate that aPC induces anergy and metabolic quiescence and aPC ameliorates experimental GvHD in mice by altering T cell plasticity.

P.C3.01.13

In vitro-generated Th9 cells mediate anti-tumor cytotoxicity against B cell lymphomas in the absence of graft-versus-host disease (GVHD)

T. Reisser[1], *I. Knape*[1], *J. Scheurer*[1], *F. Leithäuser*[2], *K. Debatin*[1], *G. Strauss*[1];
[1]*University Medical Center Ulm, Department of Pediatrics and Adolescent Medicine, Ulm, Germany,* [2]*Institute of Pathology, University of Ulm, Ulm, Germany.*

Introduction: T helper 9 cells (Th9) are characterized by the massive production of IL-9 and contribute to immunopathologies in autoimmunity, but mediate immune responses against helminth infections and solid tumors. GVHD is the most frequent and severe complication after allogeneic bone marrow transplantation (BMT) induced by mature donor-derived T cells destroying recipient target organs.
Methods: We explored in different MHC-mismatched BMT models, whether in vitro-generated Th9 cells influence GVHD development and whether they exhibit a graft-versus-tumor (GVT) effect. Therefore, allogeneic in vitro-generated Th9 cells were co-transplanted with bone marrow cells and different hematological tumor cell lines.
Results: 5 days of Th9 differentiation from naïve CD4 T cells with TGF-ß, IL-4, anti-IFN-γ and TL1A achieves >60 % IL-9$^+$ TNF-α$^-$ IFN-γ$^-$ cells distinguishable by their cytokine profile from other Th-subsets. In different BMT models, in vitro-generated Th9 cells did not induce clinical and histological GVHD or systemically increase the GVHD-associated cytokines TNF-α and IFN-γ. Th9 cells migrated to but did not attack GVHD target organs, but changed their phenotype into IL-9$^-$ TNF-α$^+$ IFN-γ$^+$ producing cells. Regarding the GVT effect, Th9 cells could efficiently prevent tumor development of B cell lymphomas but did not exhibit anti-tumorigenicity against mastocytomas or thymomas.
Conclusion: Adaptively transferred Th9 cells elicit efficient anti-tumorigenicity against different B cell lymphomas while other tumor entities are not recognized. Th9 cells might therefore have the potential to serve as therapy in BMTs to eliminate residual tumor cells. Future experiments will elucidate the molecular mechanism of Th9 selectivity towards B cell malignancies.

P.C3.01.14

Minor histocompatibility antigens as cell therapy target and GvHD predictor after allogeneic hematopoietic stem cell transplantation

D. Romaniuk, *A. Postovskaya, A. Khmelevskaya, N. Bykova, D. Malko, G. Efimov;*
National Research Centre for Hematology, Moscow, Russian Federation.

Use of fully HLA-matched donor for HSCT does not completely prevent development of graft versus host disease (GvHD), which could be driven by minor histocompatibility antigens (MiHA). MiHAs are polymorphic peptides, presented in context of HLA. When MiHA coding gene expression is restricted to the hematopoietic lineage a mismatch could lead to selective graft-versus-leukemia (GvL) reactivity, lowering the risk of relapse. MiHA genotyping of donor-patient pairs is a promising approach to predict severe side effects and to select targets for immunotherapy. We designed multiplex qPCR MiHA genotyping method, and genotyped 25 pairs (10 siblings) for 20 antigens presented in HLA-A*02:01. Related and unrelated pairs have 1-5 (median 2) and 2-7 (median 4) mismatches (MM) respectively. Using expression data from Human Protein Atlas we separated MiHA-coding genes to predominantly hematopoietic and ubiquitous and assigned GvHD/GvL prediction scores to patients. Patients with grade III acute GvHD got up to 7 MM, grade II - 4 and grade I - 3. We analyzed CD8+ T cell response specific to HA-1 and HA-2 using MHC tetramers in six mismatched pairs. We were able to detect antigen-specific response to both HA-1 and HA-2 by cell culture assays only in one patient, who developed grade III aGvHD. Sequencing of TCR-beta repertoires demonstrated the polyclonal nature of both responses. Overall the data suggests that MiHA genotyping of donor-patient pairs represent a valid strategy for GvHD studies, and could be used to search therapy targets. This work has been supported by Russian Foundation for Basic Research grant 17-04-02186.

P.C3.01.15

Long term complete remission after haploidentical allogeneic hematopoietic stem cell transplantation in a 62-year-old man with donor cell myeloid leukaemia and multiple myeloma after allogeneic hematopoietic stem cell transplantation for the treatment of chronic myeloid leukemia

***S. Sánchez Alonso**, A. Alcaraz, J. L. Steegman, Á. Figuera, A. Alegre, C. Muñoz;*
Hospital Universitario de La Princesa, Madrid, Spain.

Second malignancies are a well-defined late complication after hematopoietic stem cell transplantation (HSCT) which includes myelodysplastic syndrome (MDS) and acute myeloid leukemia developed de novo in engrafted cells of donor origin. These donor cell-derived myelodysplastic syndrome (DCM) and donor cell-derived leukemia (DCL) are intriguing phenomena which are considered to carry an extremely poor prognosis, consistent with other secondary leukemias. Intuitively, a different mismatched donor would seem the best therapeutic option since significant graft-versus-leukemia effect would not be anticipated in the total absence of HLA disparity between donor T cells and the leukemic clone.
This case report describes a 62 years old man diagnosed of Philadelphia chromosome positive chronic myeloid leukaemia in chronic phase which relapsed after 20 years from receiving an allogenic HLA-identical sibling HSCT transplantation while maintaining a complete donor chimerism. The patient developed a DCM and a donor-cell monoclonal gammopathy of undetermined significance (MGUS), which finally progress to a myeloid DCL and a smoldering multiple myeloma, respectively. Interestingly, in the meantime his donor also developed a MDS and an MGUS with the same paraprotein isotype as the recipient, which support the existence of a primary inherited gene defect constituting one of the very first hits in the pathogenesis of blood cancers.
The patient was successfully treated with HSCT from his son and remains in complete remission to the date after 33 months, which supports the hypothesis that a haploidentical HSCT is the more logical consolidation strategy in donor cell-derived haematological malignancies.

P.C3.01.16

Rapamycin increases myeloid-derived suppressor cell (MDSC) activation and immunosuppressive function in protecting against graft-versus-host disease (GVHD) in a murine allogeneic bone marrow transplantation (BMT) model

***J. Scheurer**[1], T. Reisser[1], I. Knape[1], F. Leithäuser[2], K. Holzmann[3], K. Debatin[1], G. Strauss[1];*
[1]Department of Pediatrics and Adolescent Medicine, 89075 Ulm, Germany, [2]Institute of Pathology, 89075 Ulm, Germany, [3]Genomic-Core Facility, 89075 Ulm, Germany.

Introduction and Objectives: MDSCs are a heterogeneous population of myeloid progenitors, which suppress T-cell functions and expand during inflammation. GVHD also induces MDSCs, which however don´t protect from GVHD-induced death. Rapamycin is a macrolide immunosuppressant with protective effects against GVHD. The effect of Rapamycin on MDSC in GVHD regulation isn´t well defined. We explored the effect of Rapamycin (1) in GVHD prevention, (2) on MDSC-induction, activation and immunosuppressive function and (3) on changes in T-cell function, polarization and exhaustion.
Methods: Allogeneic bone marrow transplanted mice were treated with Rapamycin or PBS every 2nd day. MDSCs were isolated from spleens and GVHD target organs for defining transcriptional changes and their immunosuppressive capacity. Allogeneic T-cells were analyzed for changes in the expression of cytokines, cytotoxic molecules and exhaustion markers.
Results: Rapamycin treatment effectively prevented clinical and histological GVHD and increased survival. Rapamycin increased splenic MDSC numbers and enhanced their suppressive potential towards alloreactive T-cells. Rapamycin-activated MDSCs significantly up-regulated iNOS expression, while immunosuppressive factors arginase-1, HO-I, COX-2 and IDO were unchanged. Transcriptome analysis revealed distinct transcriptional changes in Rapamycin-activated MDSCs, most evident for genes involved in innate and inflammatory responses, and in the regulation of secretion. Despite Rapamycin induced MDSC-activation, no alterations in T-cell exhaustion, Th1/Th2 polarization or expression of cytotoxic molecules were detectable.
Conclusion: Rapamycin is an effective adjuvant for GVHD prevention, which induces and activates highly immunosuppressive MDSCs. Further experiments will clarify, whether Rapamycin treatment maintains the graft-versus-tumor effect, a major goal in allogeneic BMT therapy.

P.C3.01.17

Non-hematopoietic Antigen Presenting cells in Lymphoid organs

***H. Shaikh**[1,2], M. Qureischi[1,2], K. Ottmüller[1,2], M. Otto[1], D. Le[1,2], A. Beilhack[1,2];*
[1]Department of Internal Medicine II, University Hospital Würzburg, Würzburg, Germany, [2]Graduate School of Life Sciences, Julius-Maximilians University Würzburg, 97078 Würzburg, Germany.

Hematopoietic stem cell transplantation is a curable therapy for hematopoietic malignancies. Allogeneic T-cells residing within the graft have potential to eliminate remaining malignant cells by Graft versus leukemia effect. Nevertheless, in 30–60% of HSCT cases allogeneic T-cells become alloreactive and attack host tissues resulting in acute Graft versus host disease. Dendritic cells are professional antigen presenting cells (APCs) and have thought to be the prime candidate for presenting self-antigens to allogeneic T-cells. However, recent findings claim that even in absence of DCs and other hematopoietic APCs GvHD cannot be prevented, suggesting role of non-hematopoietic APCs in the activation of allogeneic T-cells.
In this project, we investigate role of lymph node stromal cells (LNSCs) in initiation phase of aGvHD and their potential role as non-hematopoietic APCs.
We show that T-cells are activated in lymphoid organs and not in GvHD target organs. In MHC-II deficient bone marrow chimeras bearing MHC-II$^+$ non-hematopoietic APCs, allogenic T-cells still get activated but to a lesser extent. Furthermore, LNSCs upregulates co-stimulatory receptors early after conditioning suggesting their contribution as active antigen presentation cells in initiation phase of aGvHD. However, LNSCs significantly downregulate MHC-II on their surface as LNSCs have been shown to acquire MHC-II from hematopoietic APCs. In line with this, we observed that post irradiation DCs depletion results in loss of MHC-II on LNSCs.
Conclusively, we show that alloreactive T-cells are activated within SLOs even in absence of professional hematopoietic APCs, but still it remains elusive, which non-hematopoietic cell initiates alloreactive T-cell response.

P.C3.01.18

Unraveling the minor histocompatibility antigen immunogenicity through naive CD8 T cell frequencies estimation

***S. Sheetikov**[1], A. Kuchmiy[2], D. Romaniuk[1], G. Efimov[1];*
[1]National Research Center for Hematology, Moscow, Russian Federation, [2]Ghent University, Ghent, Belgium.

Allogeneic bone marrow transplantation is the potentially curative treatment for malignant hematopoietic disease. The therapeutic effect depends on the immune response of donor lymphocytes to residual tumor cells (graft-versus-leukemia effect). At the meantime broader immune response of transplant towards recipient could be detrimental (graft-versus-host disease). In HLA-matched transplantation donor's lymphocytes recognize the differences in peptides repertoires displayed within MHC as foreign antigens (minor histocompatibility antigens). The magnitude of immune responses determines the development of graft-versus-host disease or graft-versus-leukemia reactivity. Different minor histocompatibility antigens differ in their immunogenicity in vivo. Our hypothesis is that this can be explained by the unequal frequency of naive antigen-specific T cells in the donor repertoire. In the current study we examined the size of the naive T cell pool specific for antigens HA-1, HA-2, ACC-1Y, LB-ADIR-1F and LB-NDC80-1P. Donors were genotyped for minor antigens and HLA, and selected on the basis of having restricting HLA allele and lacking allele encoding the minor antigen of interest. By culturing naive CD8 T cell with dendritic cells loaded with minor antigen peptides we expanded and detected rare antigen-specific clones and estimated their frequencies with limiting dilution approach. Our results show that the incidence of naive precursors specific for the LB-ADIR-1F($f_{LB\text{-}ADIR\text{-}1F} \approx 3.5*10^{-6}$), HA-1($f_{HA\text{-}1} \approx 5*10^{-6}$) and HA-2 antigens($f_{HA\text{-}2} \approx 5*10^{-6}$) is significantly higher, than for LB-NDC80-1P($f \approx 2*10^{-7}$) and ACC-1Y($f \approx 2*10^{-7}$) antigens, which corresponds to their immunogenicity in vivo. This data can be useful for selection of the donor and immunosuppressive regimen and for the graft design. Funding was provided by Russian Science Foundation grant 17-15-01512.

P.C3.01.19

Expression of CD38 is elevated on peripheral blood lymphocytes of patients that do not develop chronic GvHD after HSCT

***D. Tomaz**[1], R. Ellis[2], N. Petrov[2], S. Heck[2], G. J. Mufti[1,3], V. Mehra[3], S. Kordasti[1], V. Potter[3], L. D. Barber[1];*
[1]School of Cancer and Pharmaceutical Sciences, King's College London, London, United Kingdom, [2]National Institute of Health Research Biomedical Research Centre, Guy's Hospital, London, United Kingdom, [3]Department of Haematological Medicine, King's College Hospital, London, United Kingdom.

Chronic graft-versus-host disease (cGvHD) is a significant cause of morbidity and mortality following allogeneic haematopoietic stem cell transplantation (allo-HSCT). CD38 is associated with immunosuppressive activity, but its potential role in the context of cGvHD is unknown. In this study, we performed a comprehensive deep-immunophenotyping mass cytometry analysis of peripheral blood lymphocytes from nine patients with cGvHD and eight patients without cGvHD after allo-HSCT using alemtuzumab for lympho-depletion, and eight healthy volunteers. PhenoGraph clustering tools were used for data analysis. Patients with cGvHD were found to have a paradoxically higher frequency of CD4+ Treg cells compared to those without cGvHD (p=0.018). cGvHD patients had a higher frequency of exhausted phenotype (FoxP3low, CD45RAnegative) CD4+ Treg cells compared to patients without cGvHD (p=0.028), who had a higher frequency of activated phenotype (FoxP3high, CD45RAnegative) CD4+ Treg cells (p=0.032). Notably, we observed that the frequency of CD38+ Treg cells and expression levels of this marker were significantly elevated in patients without cGvHD compared to those with active cGvHD. Moreover, expression of CD38 was also significantly elevated in CD4+ T cells, CD8+ T cells and NK cells in patients without cGvHD. In conclusion, the activated CD4+ Treg phenotype and comparatively higher expression of CD38 on lymphocytes in allo-HSCT patients without cGvHD suggests an increased ability to modulate allo-immune responses.

P.C3.01.20

Antigen experienced subtypes of cytomegalovirus specific T-cells substantially differ in clonality

***M. Vagida**, N. Bykova, G. Efimov;*
National Research Center for Hematology, Moscow, Russian Federation.

Cytomegalovirus infection is a serious complication after allogeneic hematopoietic stem cell transplantation. One of the promising therapeutic strategies is adoptive transfer of virus specific T-lymphocytes. Central memory cells have more proliferative potential than other memory subtypes and might be more applicable for therapy. In this work, we sorted three populations of CD8+ lymphocytes, specific for HLA-A02 immunodominant cytomegalovirus epitope - NLV: effector memory, terminal effectors and central memory. RNA was extracted and used as template for T-cell receptor library amplification with unique barcode adapters attached to each cDNA molecule. DNA library was sequenced on the Illumina platform.Studied T-cell subpopulations drastically vary in the diversity of T-cell receptors. The ratio of unique clonotypes to total cells was much higher for central memory T cells 0.349 than 0.127 and 0.022 for effector memory and terminal effectors respectively. Terminal effector repertoire is extremely contracted, compared with repertoires from two other subpopulations. This effect could be a result of proliferation only of small numbers of clones during differentiation to terminal effector T cells. The high number of unique clonotypes in central memory fraction may be the result of a smaller niche and the absence of expansion in the process of differentiation from naive cells. The difference in diversity of T-cell receptor repertoires of CD8+ virus specific T-cell subsets may result in differed therapeutic potential of these cells.This work was supported by the Russian Foundation for Basic Research grant 17-04-02088.

P.C3.02 Regulatory Mechanisms in Transplantation

P.C3.02.01

Use of a chimeric antigen receptor-like HLA molecule in T cells for the elimination of anti-HLA alloantibody producing B cells

***S. Betriu Méndez**[1], J. Rovira[1], B. Marzal[1], E. Banon-Maneus[1], A. Mulder[2], F. Claas[2], M. Juan[1], F. Diekmann[1], E. Palou[1];*
[1]Fundació Clínic, Hospital Clínic de Barcelona, Barcelona, Spain, [2]Academic Hospital Leiden, Leiden, Netherlands.

Introduction: Chimeric antigen receptor (CAR) T cell therapy has emerged as a very promising approach to combating cancer with excellent results in anti-leukemic clinical trials, and in a broader appraisal, promising immunotherapeutic results. A major problem in solid organ transplantation is the presence and the generation *de novo* in the recipient of donor specific antibodies which preclude the success of the transplant due to the associated high risk of antibody-mediated rejection. B cells contribute in the acute and chronic allograft rejection processes with the production of anti-donor HLA antibodies. We hypothesize that a CAR (chimeric antigen receptor)-like molecule with a particular HLA molecule as the CAR extracellular domain will engineer T cells to kill alloimmune B cells with anti-HLA antibodies as BcR, completely eliminating alloantibodies in a specific manner.
Methodology and Results: First step, we cloned the extracellular region of HLA-A*02:01 from A*02:01 positive donor cDNA by PCR. We constructed CARs with this extracellular domain and 4-1BB /CD3ζ intracellular signalling domains and delivered into human T cells by lentiviral transduction. Next, to evaluate the specificity and functionality of CAR T cells, we performed a cytotoxicity assay by co-culturing them with anti-HLAA2 alloantibodies producing EBV-transformed B cells.
Conclusion: HLA CAR-like receptor has been constructed in our laboratory and used to generate specific T cells directed to kill anti-HLA alloantibody producing B cells. This technology could open new ways of treatment and prevention of antibody-mediated rejection in solid organ transplantation.

P.C3.02.02

Reconstituted allogenic immune cells direct resistance to tumors in mice

***N. Dang**, Y. Lin, O. Rutgeerts, A. D. Billiau, M. Waer, B. Sprangers;*
KU Leuven, Leuven, Belgium.

Abstract: Acute graft-*versus*-host disease (aGVHD) is an often-lethal syndrome resulting from immune reconstitution and cytotoxic T cells (CTLs) response to allogenic bone marrow transplantation (BMT). Regulatory T cell (Treg) mediated-tolerance is a defense strategy against aGVHD that has a direct positive impact on resistance to tumor - graft-*versus*-tumor (GVT) effect. Here, we demonstrate that induction of Treg cells in response to tumor milieu is critical to establish tolerance to aGVHD after T cell-replete BMT. The protective effect of donor-derived Treg cells is exerted via a mechanism that counters Teff/Treg ratio, and in doing so, M2 polarization as a result of relatively limited tumor-associated macrophages (TAMs). This is required to prevent the development of aGVHD that otherwise promotes resistance to tumor. The reduction of Treg cells establishes the GVT effect therapeutically after T cell-depleted (TCD) BMT. In conclusion, the GVT effect of TCD-BMT relies on a crosstalk between host tumor milieu and *de novo* CTLs response, required to expand TAMs compatible with M1 polarization. **Significance:** Tumor-driven host Treg cells promotes graft acceptance and expansion of donor TAMs that induce *de novo* CTLs after TCD-BMT, rather than donor-derived Treg cells after T cell-replete BMT, an immune reconstituted effect that confers either GVT effect or host tolerance to aGVHD, respectively.

P.C3.02.03

Preservation with portable ex vivo lung perfusion (EVLP) reduces significantly ischemia/reperfusion injury in lung recipients by promoting cytokine antagonists

***C. S. Falk**[1], B. Wiegmann[2], R. Bellmàs Sanz[1], C. Neudörfl[1], C. Kühn[2], I. Tudorache[2], M. Avsar[2], A. Haverich[2], G. Warnecke[2];*
[1]MHH Institute of Transplant Immunology, Hannover, Germany, [2]MHH Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover, Germany.

Objectives: The INSPIRE trial revealed significant reduction of PGD grade 3 using the Organ Care System (OCS) compared to the standard of care (SOC) for lung preservation. In order to investigate immunological mechanisms during these preservations, immune mediators were measured with the hypothesis that OCS preservation supports an anti-inflammatory milieu leading to reduced ischemia/reperfusion injury (IRI). Methods: Plasma, perfusates from 33 patients with OCS- vs. 26 with SOC-preserved lungs were analysed for 95 immune mediators using multiplex assays. Recipient demographics, cold ischemic times (CIT), PGD scores at T0, T24 were assessed. Results: Clinical evaluation (OCS/SOC) revealed equal distributions of recipient age and diagnoses. Cold ischemic time (549 vs. 258min) was significantly shorter in OCS group ($p<0.0001$). Improved PGD score was observed in OCS recipients ($p=0.035$). IL-6, CXCL8-10, plasma levels were reduced in OCS patients at T0 ($p<0.01$). IL-6 plasma levels of SOC recipients correlated with CIT, PaO2/FiO2 ratio and PGD >2 (all $p<0.01$). However, higher levels were observed in OCS vs. SOC perfusates ($p<0.001$). We proposed suppression of IRI by induction of antagonists. IL-1RA was significantly higher in OCS perfusates ($p<0.05$) and IL-31 correlated with IFN-g ($p=0.001$) only in OCS perfusates. Conclusion: Recipients of OCS-preserved lungs show significantly reduced IRI by reduced levels of pro-inflammatory immune mediators. Strong correlation of IL-6 with CIT and PGD in SOC but not OCS patients argues for its impact on IRI and early lung function. Thus, EVLP may have the potential to ameliorate IRI and improve clinical outcome by induction of cytokine antagonists.

P.C3.02.04

Silencing MHC expression on the organ's endothelium decreases its immunogenicity and prevents a pro-inflammatory cytokine response

***C. Figueiredo**[1], M. Carvalho-Oliveira[1], C. Chen-Wacker[1], Y. Yuzefovych[1], K. Hoeffler[2], O. Pogozhykh[1], Z. Jin[1], A. Haverich[2], G. Warnecke[2], R. Blasczyk[1];*
[1]Institute for Transfusion Medicine, Hannover Medical School, Germany, Hannover, Germany, [2]Department of Cardiac-, Thoracic-, Transplantation- and Vascular Surgery, Hannover Medical School, Hannover, Germany, Hannover, Germany.

Introduction: HLA mismatches are the main cause of graft rejection and failure. Methods: SLA class I and II expression was silenced on the lung endothelium using short hairpin RNAs-encoding lentiviral vectors. The effect of silencing MHC expression was evaluated in a porcine lung transplantation model by monitoring the cytokine response during 12 weeks. Immunosuppression was stopped 4 weeks after transplantation. SLA expression was silenced during normothermic ex vivo perfusion. Nanoluciferase was used as a reporter gene. Levels of SLA were quantified by RT-PCR and flow cytometry. Cytokines were monitored every second day after Tx and weekly after the post-operative day (POD) 7 using multiplex technology. Results: SLA downregulation on lung endothelial cells reached a level of 70%. Already 1h after Tx the serum levels of IL-1beta, IL-6 and IL-8 increased significantly in all animals by up to 0.263, 1.370 and 0.497 pg/ml, respectively.
On POD 1, the cytokine secretion in the SLA silenced group decreased to pre-transplant levels whereas those of the control group remained significantly elevated ($p<0.01$). On POD 14, levels of IL-12 increased significantly by up to 0.286 pg/ml in the control group whereas it remained at pre-transplant levels in the SLA silenced lung recipients. In addition, levels of IL-2, IL-10 and TNF-α increased exclusively in animals with SLA expressing lungs while it was undetectable in animals receiving SLA silenced lungs. Conclusion: These data indicate that MHC-silenced grafts are less immunogenic and may combat the burden of rejection and immunosuppression.

P.C3.02.05

Liver resident mucosal-associated invariant T (MAIT) cells display an active but less proliferative state compared to the peripheral MAIT cells

***W. Huang**, W. He, X. He, Y. GAO;*
The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China.

The liver is one of the most important immunological organs that remains tolerogenic in homeostasis. The composition of leukocytes in the liver is highly distinct from that of the blood and other lymphoid organs. In particular, the enrichment of innate T cells, i.e. invariant NKT cells (iNKT cells), Mucosal Associated Invariant T cells (MAIT cells).
We performed flow cytometry on MAIT cells in blood and perfusion fluid from 20 patients undergone liver transplantation. Further functional assessments including MAIT cell proliferation, activation, and cytokine-producing capacity.

A much higher percentage of MAIT cells were detected in the perfusion fluid compared with those in the PBMC (8.22% vs. 2.7%, p<0.001). In addition to that, the PBMC peripheral MAIT cells were in a less active state (with lower CD69 expression) compared to their perfusate counterpart. Cytokines productivity post IL-12/IL-18 stimulation by MAIT cells were similar in both peripheral and perfusate as measured by the Luminex analysis. Interestingly, the Liver MAIT cells were found to be less proliferative upon stimulation with anti-CD3/CD28.
In here, we demonstrated that MAIT cells are numerically rich in the liver and functionally distinctive compared to their peripheral counterpart. These observations may be significant in transplantation tolerance in the liver and other liver diseases.

P.C3.02.06
Long-term graft tolerance induction by NFATc pathway inhibition in innate immune cells

L. Gornati[1], *C. Cigni*[1], *F. Mingozzi*[1], *L. Marongiu*[1], *R. Rotem*[1], *M. Colombo*[1], *D. Prosperi*[1], *I. Zanoni*[1,2], *F. Granucci*[1];
[1]*Department of Biotechnology and Bioscience, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milan, Italy, Milan, Italy,* [2]*Harvard Medical School and Division of Gastroenterology, Boston Children's Hospital, Boston, MA 02115, USA, Boston, United States.*

Introduction: Transplantation is a therapeutic approach for failing organs, whose most feared complication is rejection, mediated by the instauration of alloreactive T cells, supported by the Nuclear Factor of Activated T cells (NFAT). To avoid graft loss, transplanted patients undergo life-long administration of immunosuppressive drugs, completely abrogating the immune response. Since NFAT has recently emerged to be pivotal in sustaining innate immune cells, we aim at evaluating NFAT as a potential specific therapeutic target in these cells to prevent rejection without causing complete immune paralysis.
Methods: By taking advantage of nanoparticles (NPs) delivering a specific NFAT inhibitor peptide to innate immune myeloid cells, we managed to abolish NFAT activation in these cells without affecting T cells, in a model of mismatched skin graft.
Results: The administration of our NPs prevents acute allograft rejection. Accordingly, transplanting NFATc2 KO skin, results in delayed rejection, due to the impaired vessels permeability and thus migration of T cells to the graft as well as of dendritic cells (DCs) to the draining lymph nodes. Moreover, we noticed a strong increase of $CD4^+$ $CD25^+$ $Foxp3^+$ T cells in NPs-treated recipients, suggesting regulatory T cells-mediated tolerance against the graft. Accordingly, anti-NFAT NPs administration can be interrupted without provoking graft rejection. Diversely, if animals are treated with tacrolimus to inhibit rejection, therapy interruption immediately results in graft loss.
Conclusions:
The inhibition of the NFAT signaling pathway in innate immune cells can be considered as a new approach to induce long-term graft acceptance without affecting T cell functions.

P.C3.02.07
Induction and effector phase of human anti-pig T cell responses are differentially affected by the CRISPR/Cas9 induced absence of porcine MHC/SLA class-I molecules

R. Hein, *S. Clever, H. Düvel, B. Trautewig, A. Brinkmann, J. Hundrieser, R. Schwinzer;*
Transplantation Laboratory, Clinic for General, Visceral and Transplantation Surgery, Hannover, Germany.

Introduction: Foreign MHC molecules display the main target for induction of anti-graft responses and rejection processes in the T-cell effector phase. By genetic engineering it is possible to generate porcine cells and tissues lacking MHC expression which might particularly be suited for clinical xenotransplantation. Thus, we asked how the absence of porcine MHC/SLA class-I (SLA, "swine leucocyte antigen") affects the human anti-pig immune response. Therefore, a SLA class-I negative porcine cell line was generated and its stimulatory capacity to activate human T-cells was characterized *in-vitro.*
Methods: SLA class-I deficient B-cell line L23 (porcine APC, $SLA\text{-}I^+$, $SLA\text{-}II^+$) was generated using the Cas9 nuclease and a guide RNA directed against the beta2-microglobulin (b2m) coding gene.
Results: In flow cytometry analyses no b2m or SLA class-I expression was detected in L23-b2m-ko cells; expression of SLA class-II and costimulatory molecules (CD40, CD80/86) was comparable to wildtype (wt) L23 cells. Proliferation of hPBMC triggered by L23-b2m-ko cells was significantly reduced compared to L23-wt stimulation, mainly due to poor reactivity of $hCD8^+$ T-cells. However, ^{51}Cr-release assays showed no protection of SLA class-I negative cells against cell-mediated lysis by human L23-specific cytotoxic T cells (generated in 6-day MLRs between hPBMC and L23-wt cells): L23-b2m-ko cells were lysed with similar intensity as L23-wt cells.
Conclusion: The induction phase of human anti-pig T cell reactivity can be diminished by the elimination of porcine SLA class-I molecules. However, alternative strategies will be required to protect porcine cells from human cytotoxic effector cells.
Supported by the DFG (SFB-TRR 127 "Xenotransplantation").

P.C3.02.08
In vivo and in vitro modulation of mTOR pathway enhances human γδ T cells effector functions

H. Kaminski[1], *V. Pitard*[2], *L. Couzi*[1,3], *P. Merville*[1,3], *A. Tarricone*[4], *C. Larmonier*[4], *I. Pellegrin*[4], *R. Duran*[5], *J. Déchanet-Merville*[1];
[1]*CNRS-UMR 5164 Immunoconcept, Bordeaux University, bordeaux, France,* [2]*CNRS-UMR 5164 Immunoconcept, bordeaux, France,* [3]*renal transplant department, bordeaux university hospital, Bordeaux, France,* [4]*Immunology department, Bordeaux university hospital, bordeaux, France,* [5]*INSERM UMR 1218, Bordeaux University, bordeaux, France.*

Being able to control γδ T cell effector functions is important for the development of γδ T cell-based immunotherapy. mTOR pathway enhances glycolytic metabolism in effector T cells and is crucial for cytotoxicity and IFNγ production. mTOR inhibitors (mTORi) are used in transplantation to prevent rejection but are also paradoxically associated with less CMV infections, the most frequent adverse event of immunosupression. As Vδ2neg γδ T cells are key players against CMV, we wondered if mTORi are able to modulate γδ T cell functions in transplant patients. *In vitro*, Vδneg γδ T cells from CMVpos transplant recipients surprisingly expanded better in the long-term (3 weeks) presence of clinical mTORi doses. They also displayed higher S6 and AKT phosphorylation and higher T bet production, as well as increased IFNγ production when incubated with CMV-infected cells. Our working hypothesis is therefore that mTORi could inhibit mTORC1 while inducing a negative feedback increase of AKT phosphorylation, leading to phosphorylation of S6 and expression of T bet and IFNγ. *In vivo*, faster CMV-induced Vδneg γδ T cell expansion was observed in patients treated with mTORi versus mycophenolate mofetil. Transcriptomic analyses of purified Vδneg γδ T cells from mTORi-treated patients showed higher transcription of the glycolytic enzyme GPI, of IL2Rbeta, CD44, and NFkB, all signs of γδ T cell activation. To conclude, mTORi could increase antiviral functions of γδ T cells in transplant patients and be used in immunotherapy to promote γδ T cell effector functions against tumors or infections.

P.C3.02.09
Modulation of the IL-33-ST2 axis in regulatory T cell therapy

K. Kawai, *M. Uchiyama, J. Hester, F. Issa, K. J. Wood;*
University of Oxford, Oxford, United Kingdom.

Background: Regulatory T cells (Tregs) are crucial mediators of immune homeostasis, with the ability to modulate alloreactive T cell responses and control transplant rejection. Previous work has demonstrated that the modulation of the interleukin-33 (IL-33)/ST2 axis expands a highly suppressive subpopulation of Tregs. Here we present novel data that demonstrate the ability of exogenous IL-33 administration to expand mouse Tregs *in vivo* that can promote the survival of MHC-mismatched skin grafts.
Materials/Methods: Groups of mice received either saline or recombinant IL-33 (1μg/d for 6 days). $CD4^+FoxP3^+$ Tregs from control or IL-33-treated mice were then flow sorted and adoptively transferred together with effector T cells (Teffs) into syngeneic immunodeficient mice. Mice were then transplanted with an allogeneic skin graft where survival was monitored until allograft rejection and their organs were harvested for phenotypic analysis.
Results: Recombinant IL-33 administration expanded $CD4^+FoxP3^+$ Treg populations 10X *in vivo* (p<0.0001). Mice treated with sorted IL-33-expanded Tregs demonstrated an enhanced ability to modulate Teff responses and suppress allograft rejection. While mice treated with Teff only rejected their allografts as expected, with a median survival time (MST) of 14 days, naive and IL-33 Treg administration improved survival to an MST of 64 days and >100 days, respectively. Conclusion: Administration of IL-33 *in vivo* can significantly expand Tregs, which demonstrate an enhanced ability to prolong allogeneic skin grafts *in vivo*. These data highlight an important pathway for the modulation of Treg therapy, which may allow the use of fewer cells in the future.

P.C3.02.10
BIUxx

K. Kotschwarova, *M. Fialova, V. Svachova, L. Curnova, K. Vlasakova, O. Viklicky, I. Striz;*
IKEM, Prague, Czech Republic.

Proinflammatory cytokines induced by ischemia/reperfusion injury upregulate release of chemokines from parenchymal cells to attract immune cells into transplanted kidney. The aim of our study was to evaluate the ability of human renal proximal epithelial cells (RPTEC) to produce multiple chemokines in response to TNF-α and compare the data with chemokine induction in renal adenocarcinoma (RA) and monocyte/macrophage (THP-1) cell lines. Concentrations of CXCL1, CXCL4, CXCL5, CXCL8, CXCL9, CXCL10, CXCL11, CXCL16, CCL2, CCL5, CCL18, CCL21 and CX3CL1 were measured in culture supernatants by Luminex. Chemokines attracting neutrophils (CXCL1, CXCL5, CXCL8), were produced preferentially by TNF-α stimulated epithelial cells. RPTEC released more CXCL1 and CXCL8 while RA cells produced preferentially CXCL5. RPTEC and RA are also an important source of CCL2, chemotactic for monocytes and other immune cells. In contrast, TNF-α stimulated THP-1 cells produced more CCL5 (specific for monocytes, eosinophils and Th cells).

Chemokines attracting Tc and NK cells (CX3CL1, CXCL16) and activated T cells (CXCL10, CXCL11) were preferentially induced by TNF-α in RPTEC. We conclude that chemokines released from human renal epithelial cells in response to TNF-α do not regulate only the influx neutrophils but are involved also in the recruitment of effector cell populations of adaptive immunity. Our data suggest, that primary epithelial cells are more convenient for studying chemokine regulation in kidney than renal adenocarcinoma epithelial cell line. Supported by Ministry of Health of the Czech Republic, grant NR 15-26883A and by MH CZ-DRO (Institute for Clinical and Experimental Medicine - IKEM, IN 00023001).

P.C3.02.11

A novel way to generate human HLA class II monoclonal antibodies for HLA epitope analysis

C. S. M. Kramer[1], *M. E. Franke-van Dijk*[1], *C. C. Zilvold-van den Oever*[2], *R. Rademaker*[2], *P. W. Parren*[1], *D. L. Roelen*[1], *S. Heidt*[1];
[1]*Leiden University Medical Centre, Leiden, Netherlands,* [2]*Genmab, Utrecht, Netherlands.*

Donor-specific antibodies produced upon renal transplantation are triggered by immunogenic epitopes present on mismatched donor HLA. However, not every foreign epitope will lead to antibody formation, which highlights the need to experimentally verify truly immunogenic epitopes. Human HLA-specific monoclonal antibodies (mAbs) have proven to be very useful for identification of HLA class I epitopes. Since currently the number of available HLA class II mAbs is limited, we aimed to produce these mAbs by using HLA class II-specific tetramers and recombinant technology, of which here we show proof of principle. From PBMCs of an individual with HLA-DRB1*07:01-specific serum antibodies, single memory B cells positive for HLA-DRB1*07:01-specific tetramers were sorted (0.0012% of B cells). After expansion, 11/36 of B cell clones produced HLA-DRB1*07:01 antibodies, three also being reactive with HLA-DRB1*09:01. Subsequently, RNA was isolated to obtain the variable heavy and light chains to clone into pcDNA3.3 expression vectors. Analysis of variable domain sequences identified B cells with different combinations of V(D)J segments, suggesting that the isolated B cells originate from different precursors. At time of writing, we recombinantly expressed one HLA-DRB1*07:01-specific mAb and could use this to confirm $25Q_3$ as an immunogenic epitope on HLA-DR7. This study demonstrates that HLA tetramers can be used to isolate HLA class II-specific memory B cells and subsequently can be used as a source to generate recombinant mAbs. With this method a large panel of human HLA class II mAbs can be produced, to verify HLA epitopes and for functional studies on HLA class II antibodies.

P.C3.02.12

Redefining the HLA-DR11 peptide binding motif with new MS data

L. Labeur*, J. A. Collado, Y. Arribas, R. Farriol, D. Jaraquemada, M. Carrascal;*
Institut de Biotecnologia i Biomedicina-Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain.

The HLA-DRB1*1101 (DR11) allele is associated to several diseases, being a protective allele for type 1 diabetes and systemic lupus erythematosus, while in multiple sclerosis it acts as a susceptibility agent. However, the last published data defining the DR11 motif are incomplete because of the low number of analysed peptides. Therefore, our objective is to accurately define this motif using a larger number of peptides eluted from the DR11 allele.
We cultured the human EBV-transformed HLA-DRB1*1101 homozygous cell line BM21 and isolated Class II-peptide complexes by immunoprecipitation with the HLA-DR-specific B8.11.2 antibody. Peptides were eluted and then processed by MALDI and Orbitrap MS. Furthermore, we analysed the peptides previously eluted from thymus and spleen samples from $DR11^+$ donors. We thus observed that the sequences of peptides obtained from human tissue matched the motif obtained from cultured cells *in vitro*. In conclusion, contrary to what is found in the current literature, three anchor positions (P1, P4 and P6) and not four (P1, P4, P6 and P9), were defined. We found that the DR11 motif is broader than previously described, although the main characteristics are maintained. All amino acids in P1 and P4 anchor positions were hydrophobic (F, L, I, V, Y and L, V, I, A, T, F respectively). In contrast, P6 was occupied by basic amino acids (R, K and S), and P9 appeared to be irrelevant. Our data provide new information and add reliability to the current databases.

P.C3.02.13

IL-23IL-17 pathway in kidney allograft rejection

Y. Lakhoua Gorgi[1], *Y. Haouami*[1], *T. Dhaouadi*[1], *M. Jellouli*[1], *J. Abdellatif*[1], *M. Majdoubi*[1], *M. Bacha*[2], *R. Goucha*[2], *R. Bardi*[1], *T. Ben Abdallah*[1], *I. Sfar*[1];
[1]*Research Laboratory in Immunology of Renal Transplantation and Immunopathology (LR03SP01), Tunis, Tunisia,* [2]*Department of Medecine and Nephrology Charles Nicolle hospital, Tunis, Tunisia.*

T helper 17 cell (Th17) subset has been implicated in autoimmune diseases, tumor immunity and transplant rejection. In order to investigate the role of interleukin 17 (IL-17)/ IL-23 pathway in allograft outcome, intragraft expression of IL-17 mRNA and polymorphisms (SNPs) of IL-17A, IL-17F, IL-17RC and IL23R genes were evaluated with a quantification of plasma IL-17A, IL-17F, and IL-23. This study revealed that recipients with acute rejection (AR) had a significant increase in IL-17A mRNA expression levels of after transplantation compared to controls (p=0.037). Moreover, plasma IL-17A levels were significantly higher in AR group; pre-transplantation (Day-1): p=0.00022 and post-transplantation (Day7): p<10E-14. IL-17F and IL-23 plasma levels were significantly higher in AR at Day7 only (47,86 vs. 22.99 pg/ml; and 33.82 vs. 18.811 pg/ml); p=0.015 and p<10E-17, respectively. Using ROC curves, IL-17A and IL-23 plasma levels exhibited excellent sensitivities and specificities for predicting AR. Genetic study revealed no association between IL-17A, IL-17F, IL-17RC and IL23R studied polymorphisms and AR. Nevertheless, plasma IL-17F levels at both Day-1 and Day7 were significantly higher in patients carrying IL-17F-1507*C/T and *T/T genotypes comparatively to those with the wild homozygous genotype *C/C; p=0.015 and p=0.022, respectively. Besides, IL-17A mRNA levels were significantly higher in patients carrying the IL-23R*G/G genotype (1367.845) comparatively to those with *G/A genotype (180.367), p=0.042. Based on these findings, significant increase of IL-17A mRNA and protein levels in AR recipients which are genetically controlled highlights the role of this cytokine that can be a useful clinical biomarker to predict early acute renal allograft rejection

P.C3.02.14

“Off-the-shelf” mesenchymal stromal cell therapy can prolong rejection-free survival of corneal allografts in a high immunological risk transplant model by increasing regulatory cell populations

P. Lohan[1], *N. Murphy*[1], *O. Treacy*[1,2], *K. Lynch*[1,2], *M. Morcos*[1], *A. E. Ryan*[2,1], *M. D. Griffin*[1,3], *T. Ritter*[1,3];
[1]*Regenerative Medicine Institute, National University of Ireland, Galway, Galway, Ireland,* [2]*Discipline of Pharmacology and Therapeutics, National University of Ireland, Galway, Galway, Ireland,* [3]*CURAM Centre for Research in Medical Devices, School of Medicine, College of Medicine, Nursing and Health Sciences, National University of Ireland, Galway, Galway, Ireland.*

Mesenchymal stromal cells (MSC) have been shown to be potently immunomodulatory and capable of prolonging corneal transplant (CT) survival. Patients with pre-existing allo-immunity or receiving a second CT are at higher risk of rejection and represent an unmet clinical need. A high risk CT model was developed in rats by injecting the recipient with donor derived splenocytes 14 days before transplantation. Pre-sensitised CT recipients rejected significantly earlier (day 11.5) than animals which were not pre-sensitised (day 19.3) indicating they had pre-existing allo-immunity. Two pre-transplant intra-venous injections on days -7 and -1 of $1x10^6$ allogeneic (to both donor and recipient) MSC were administered. This strategy significantly prolonged CT survival with 63.6% of MSC treated animals grafts surviving until day 30 compared to 0% in the untreated group. MSC treated animals had higher proportions (13.5%) of the immunomodulatory $CD11b^+B220^+$ monocytic cells in the lungs at the time of transplantation compared to untreated animals (7.6%). At the average time of rejection in untreated animals (day 10), MSC treated animals also had a higher proportion of $CD4^+CD25^+FoxP3^+$ Tregs (7.9%) in their draining lymph node compared to untreated animals (4.4%). Finally, CD11b/c cells isolated from the lungs of naïve rats were shown to generate more Treg *in vitro* after being co-cultured with MSC than those not exposed to MSC. This work sheds light on a potential mechanism of action and underlines the utility of MSC in immunological diseases and particularly in high risk CT.

P.C3.02.15

In vitro TGF-β secretion capacity of stimulated peripheral blood cells correlates with the occurrence of opportunistic infection in liver and kidney transplantation

F. Boix, G. Gonzalez-Martinesz, R. Alfaro, I. Legaz, R. Moya-Quiles, A. Minguela, J. Pons, J. Galian, S. Llorente, ***M. Muro****;*
Immunology Service, Clinical University ´Virgen Arrixaca´ - IMIB. 30120 Murcia, Spain., Murcia, Spain.

Introduction:TGF-β has been known to act as a potent immune-regulatory cytokine, which blocks T-cell activation. It is considered as a potential target for more specific and less toxic immunosuppression and control of alloimmune-responses over the long term in transplantation In this setting, TGF-β main function after SOT remains yet unclear and is important to further characterise its role under different clinical circumstances not only for operational tolerance, but also amongst other comorbidities such as opportunistic-infection(OI).
Objective:The aim of this study was to assess the concentration of TGF-β in the supernatant of stimulated WPB in a cohort of SOT recipients (SOTr) and correlate it with the primary study outcome which was the occurrence of OI. Material and Methods:30 liver(LTr) and 31 kidney(KTr) transplant recipients as well as 15 healthy volunteers (HCs) were recruited and prospective monitored for one year in our Hospital. ELISA was carried out to calculate TGF-β concentration after WPB culture with Concanavalin A for 72 hours.
Results:Prior transplantation, SOTr showed higher TGF-β concentration compare to HCs. The stratification analysis showed that TGF-β was significantly higher in patients with OI within the first six months following transplantation. A TGF-β>363.25ng/ml in LTr and >808.51ng/ml in KTr were shown to be the most accurate cut-off values to stratify SOTr at high risk of OI. The regression model confirmed this biomarker as the main recipient risk factor for developing OI. Discussion:Our data show that the quantification of TGF-β could provide valuable information as to the occurrence of OI in LTr and KTr.

P.C3.02.16

Photoconversion of Alloreactive T Cells in Peyer's Patches: Tracking the Homing of Highly Proliferative Cells *in vivo*

K. J. Ottmüller[1,2], *Z. Mokhtari*[1], *L. Scheller*[1,2], *J. Hartweg*[1,2], *S. Thusek*[1], *D. Le*[1], *M. Ranecky*[1,2], *H. Shaikh*[1,2], *M. Qureischi*[1,2], *K. G. Heinze*[3,2], *A. Beilhack*[1,2,4];
[1]*University Hospital Würzburg, Würzburg, Germany,* [2]*Graduate School of Life Sciences, University of Würzburg, Würzburg, Germany,* [3]*Rudolf Virchow Center, University of Würzburg, Würzburg, Germany,* [4]*Department of Pediatrics, University Hospital, Würzburg, Germany.*

Marking and tracking of T cells is important to study their trafficking, which is an elementary process to all immune reactions. Only with this efficient homing, single immune cell clones can convey protection against intruders or regulate immune responses throughout the whole body. Photoconversion is a superior labeling technique for intravital application because it enables contactless time- and site-specific marking of cells in the tissue without surgically manipulating the microenvironment. However, the converted fluorescent protein may decline quickly in proliferating cells.
We demonstrate the suitability of photoconversion to tracking highly proliferating T cells from the priming site of T cell activation to peripheral target organs. Dendra2$^+$ T cells were photoconverted in the Peyer's patches during the initiation phase of acute graft-versus-host disease (GvHD). We tracked these cells through the mesenteric lymph nodes and the peripheral blood to their effector site, the small intestine, with flow cytometry and intravital two-photon microscopy.
Photoconverted alloreactive T cells preserved the full proliferative capacity and cytotoxicity against tumor cells. We quantified the trafficking cells throughout their homing route and observed that their migration in the intestinal lamina propria was retained after photoconversion.
We conclusively proved that photoconversion of highly proliferative alloreactive T cells in the Peyer's patches is an effective tool to study trafficking of alloreactive T cells under physiologic conditions and to GvHD target tissues. This technique can also be applied to the study of immune cell tracking under inflammatory and non-inflammatory conditions.

P.C3.02.17

Dendritic cells are affected by VEGF depletion leading to corneal graft acceptance

A. Schneider[1], *E. Zinser*[2], *F. Bock*[1];
[1]*Department of Ophthalmology Cologne, Cologne, Germany,* [2]*Department of Immunomodulation, Erlangen, Germany.*

The main risk factor in corneal transplantations is the presence of blood and especially lymphatic vessels in the normally avascular and immune-privileged cornea. In the murine model of high risk corneal transplantation neovascularization is induced by a sterile inflammation caused by intrastromal corneal sutures. VEGF-depletion during this inflammation prevents vessel ingrowth into the cornea, which significantly improves subsequent graft survival. Dendritic cells (DCs) are the main immune cells that are trafficked through lymphatic vessels and are responsible for antigen recognition and subsequent activation of T cell mediated rejection in transplant settings. We demonstrate that VEGF-depletion does not only act on vessel formation, but has an immune modulatory effect on DCs. Treatment of DCs with VEGFR1/R2 Trap affected costimulatory molecule expression, cytokine secretion and impaired the capacity of the cells to stimulate T cells in vitro. VEGF depletion in mice during suture induced neovascularization impairs recruitment of DCs into the cornea and weakens trafficking of DCs. In addition we could show that VEGF depletion acts in an immune modulatory manner on the DCs directly through decreased costimulatory molecule expression and subsequent induction of more potent CD4+CD25+Foxp3+ regulatory T cells.

P.C3.02.18

CD40 ligation on monocyte derived dendritic cells enhances the production of the positive complement regulator properdin

M. F. van Essen*, N. Schlagwein, J. M. Ruben, C. van Kooten, on behalf of the COMBAT Consortium;*
Div. of Nephrology and Transplant Medicine, Dept. of Medicine, Leiden University Medical Center, Leiden, Netherlands.

The important role of local complement production and activation has been demonstrated in experimental transplantation. During APC-T cells interactions, C3a and C5a contribute to T cell proliferation and cytokine production by APCs. Properdin (fP) stabilizes the C3 convertase, thereby enhancing C3a and C5a generation. We investigated the regulation and functional consequence of fP production by monocyte-derived dendritic cells (MoDCs) upon LPS stimulation or the mimic of co-stimulatory signals in T cell-APC interaction CD40L. MoDCs were stimulated for 48h with either LPS, CD40L expressing L-cells or control cells. CD40L induced a dose-dependent increase in fP production (mean 7.5-fold increase, 18 ng/ml, n=10), compared to controls (mean 2.5ng/ml). LPS stimulation increased fP levels as well (mean 15ng/ml, n=10). The combined activation with CD40L and LPS did not result in synergistic fP production, whereas this was observed for IL-10 and IL-12p70.
To explore the functional role of fP, MoDCs were treated with a siRNA-pool against fP at day 5 and additionally cultured for 48 hours with or without stimulation. At day 7, cells were harvested and co-cultured with CFSE-labelled allogeneic T cells. FP knockdown was confirmed by ELISA and exposure of immature MoDCs to allogeneic T cells led to a reduced T cell proliferation, as determined by FACS.
In conclusion, LPS and CD40 activation increases fP production by MoDCs. Since CD40L is specifically expressed on activated T cells, this introduces cognate specificity to this local regulation. This places fP, in addition to C3a and C5a, as a new player regulating APC-T cell activation.

P.C3.02.19

Mechanisms of calcification in human bio-devices

A. Paul[1], *S. Ben-Arye*[1], *L. Govani*[1], *H. Yu*[2], *I. Fellah-Hebia*[3], *M. Pascual-Gilabert*[4], *C. Costa*[5], *R. Mañez*[6], *M. Galiñanes*[7], *B. Tomaso*[8], *G. Cesare*[9], *J. Roussel*[3], *T. Le Tourneau*[10], *J. Soulillou*[11], *X. Chen*[12], *L. Iop*[13], *G. Gerosa*[13], *E. Cozzi*[14], *V. Padler-Karavani*[1];
[1]*Tel Aviv University, Tel Aviv, Israel,* [2]*Department of Chemistry, University of California-Davis,, Davis, CA,, United States,* [3]*Institut du Thorax, Department of Thoracic and Cardiovascular Surgery, University Hospital, INSERM UMR1087, Nantes, France,* [4]*IUCT (Inkemia IUCT group), Mollet del Vallès, Barcelona, Spain,* [5]*Infectious Diseases and Transplantation Division, Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain,* [6]*Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain,* [7]*6Department of Cardiac Surgery, Reparative Therapy of the Heart, Hospital Universitari Vall d'Hebron and Vall d'Hebron Research Institute, Barcelona, Spain,* [8]*Cardiovascular Regenerative Medicine Group, Department of Cardiac, Thoracic and Vascular Surgery, University of Padua, Paduva, Italy,* [9]*Avantea, Via Porcellasco 7 f, 26100, Cremona, Italy,* [10]*Institut du Thorax, Department of Cardiology, University Hospital, INSERM UMR1087, Nantes, France,* [11]*Institut de Transplantation–Urologie–Néphrologie, INSERM Unité Mixte de Recherche 1064, Centre Hospitalo Universitaire de Nantes, Nantes, France,* [12]*Department of Chemistry, University of California-Davis, Davis, CA, United States,* [13]*Venetian Institute of Molecular Medicine, Regenerative Medicine Group, Department of Cardiac, Thoracic and Vascular Surgery, University of Padua,, Padua, Italy,* [14]*Transplant Immunology Unit, Department of Transfusion Medicine, Padua University Hospital, Padua, Italy.*

Susceptibility to structural valve deterioration is one of the major drawbacks of bioprosthetic heart valves (BHVs). *N*-glycolylneuraminic acid (Neu5Gc) is an immunogenic dietary-carbohydrate antigen in humans because of inactivation of the gene encoding CMP-*N*-acetylneuraminic acid hydroxylase (*CMAH*), and all humans have circulating anti-Neu5Gc antibodies. We hypothesized that interaction of anti-Neu5Gc antibodies with Neu5Gc on BHVs could lead to immune response resulting in valve deterioration through calcification. We demonstrate Neu5Gc in both native calcified human valves as well as in calcified-BHVs, explanted from human patients, by HPLC and immunohistochemistry. Furthermore, anti-Neu5Gc IgGs were purified from native calcified human valves and explanted calcified-BHVs, further validated by a glycan microarray. In the Neu5Gc-free *Cmah*-KO mouse model, anti-Neu5Gc antibodies promoted calcium deposits in subcutaneous implanted BHV discs, both with passive transfer of affinity-purified human anti-Neu5Gc IgGs, and by active-immunization of *Cmah*-KO mice with Neu5Gc-containing glyco-nanoparticles. Thus, co-existence of Neu5Gc/anti-Neu5Gc likely mediate BHV structural valve deterioration, and in addition they have potential role in native human aortic valve calcification.

P.C3.03 Organ Transplantation, Genotyping

P.C3.03.01

High intensity de novo donor specific HLA class II ntibodies associated to renal chronic rejection

R. Alenda[1], *M. Moreno-Hidalgo*[1], *A. Balas*[1], *F. Garcia-Sanchez*[1], *L. Barea*[1], *M. Rodriguez-Ferrero*[2], *F. Anaya*[2], *J. Vicario*[1];
[1]*Centro de transfusion de la comunidad de Madrid, Madrid, Spain,* [2]*Hospital Universitario Gregorio Marañon, Madrid, Spain.*

Introduction: Development of *de novo* donor-specific anti-HLA antibodies (*dn*DSA) is associated with reduction in renal graft survival. High titer (>5000 MFI) of anti-HLA class II antibodies is frequently found in chronic rejection. The aim of the study was to analyze the incidence of dnDSA, their targets/titer, and clinical relevance. **Material and Methods:** The study included 303 renal transplants from 2010 to 2018 at a single center. Sera were tested using Lifecodes Class I and Class II SAB kits (Immucor, USA). **Results:** 44 patients (14.5%) developed *dn*DSA in 144 months as average. 5 patients developed antibodies only against class I (1'6%), 11 patients against class II (3'6%) and 28 patients against both (9'2%). The mean of the high titer reached for anti-HLA class I antibodies was 4833 MFI, anti-HLA class II DRB 11123 MFI, anti-HLA class II DQB1 10258 MFI and anti-HLA class II DQA1 9890 MFI.
Class I antibodies titer is significantly lower than class II. The mean titer of anti-HLA class II DRB4* (15600 MFI) and DQB1*02,03 (11164 MFI) antibodies was calculated, because they are considered as eplets predictors for the development of d*n*DSA. In both cases, the titer is higher than was found for all DRB,DQB1 antigens. **Conclusion:** 14.5% of the patients studied develop *dn*DSA in the follow-up. The percentage of patients with anti-HLA antibodies against class II antigens is higher, and they react with higher maximum titer than anti-HLA class I antibodies. The highest antibody response seems to be directed against immunodominat eplets.

P.C3.03.02

Relationship between adaptive NK cell markers and the incidence of cytomegalovirus infection in kidney transplant recipients

M. Ataya[1], *D. Redondo-Pachón*[1,2], *L. Llinàs*[1], *J. Yélamos*[1,3], *G. Heredia*[4], *L. Soria*[4], *J. Pascual*[1,2], *M. Crespo*[1,2], *M. López-Botet*[1,3,4];
[1]*Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain,* [2]*Nephrology S. Hospital del Mar, Barcelona, Spain,* [3]*Immunology S. Hospital del Mar, Barcelona, Spain,* [4]*Univ. Pompeu Fabra (UPF), Barcelona, Spain.*

Human cytomegalovirus (HCMV) infection has been related with an increased risk for graft loss and reduced host survival in kidney transplant recipients (KTR). HCMV infection may promote the adaptive differentiation and persistent expansion of an NKG2C+ NK cell subset. These adaptive NK cells are functionally mature, efficiently mediate specific antibody-dependent effector functions against HCMV-infected cells and display to a variable extent discrete phenotypic features, reflecting late differentiation events (e.g. CD57 and ILT2 expression; FcεRI γ chain downregulation). In a prospective study of a KTR cohort (N=122), high pre-transplant levels of NKG2C$^+$ NK cells were found associated with a reduced incidence of post-transplant HCMV viremia[1]. We have extended the phenotypic analysis by multiparametric flow cytometry in available cryopreserved pre-transplant PBMC samples from cases of the same KTR cohort. The expression of NKG2A, CD57, ILT2 (LIR-1) and FcεRI-γ was analysed in NK cells and T lymphocytes, including TCRγδ+ subsets (Vδ2+ and Vδ2-). Individually, these markers appeared unrelated with post-transplant HCMV infection. Yet, when their co-expression with NKG2C in NK cells was considered, significantly lower proportions of NKG2C+ NKG2A-, CD57+, CD57-, ILT2+, ILT2-, FcεRI γ- and FcεRI γ+ were detected in KTR developing HCMV infection (Wilcoxon test). These results, together with CMV-free survival analysis (Kaplan-Meier), further support the relationship between NKG2C+ NK cells and the risk of post-transplant HCMV infection.
[1] Redondo-Pachón et al. 2017. J Immunol 1;198(1):94-101

P.C3.03.03

Ex vivo lung perfusion affects the immunological milieu and regulatory T cells in a porcine lung transplantation model

R. Bellmàs Sanz[1], *J. Salman*[2], *T. Siemeni*[2], *K. Jansson*[2], *A. Knöfel*[2], *F. Ius*[2], *K. Höffler*[2], *A. Haverich*[2], *I. Tudorache*[2], *C. S. Falk*[1], *G. Warnecke*[2];
[1]*Institute of Transplant Immunology, Hannover Medical School, Hannover, Germany,* [2]*Department of Cardiothoracic, Vascular and Transplantation Surgery, Hannover Medical School, Hannover, Germany.*

Introduction: Ex vivo lung perfusion (EVLP) is an alternative to cold static storage for graft preservation in lung transplantation. Here, we investigate mechanisms of improved preservation using the Organ Care System (OCS) as EVLP platform, focusing on immunological changes in a porcine lung transplant model. **Methods**: 12 porcine lungs were explanted from healthy donor pigs: six lungs were preserved for 6h in the OCS (OCS group) and six lungs on ice (standard of care, SOC group). The left lungs were transplanted into allogeneic porcine recipients and all pigs were observed for 6 hours after clamping the contralateral lung. We investigated the presence of cytokines and Treg, Tcon subsets in recipient blood, bronchoalveolar lavage (BAL) fluid and perfusates. **Results**: In BAL of OCS recipients at 6 h, significantly lower levels of IL-6 (p=0.04), IL-1a (p=0.04), IL-1RA (p=0.03), IL-12 (p=0.03), IL-18 (p=0.03) and IFN-g (p=0.04) were observed compared to SOC recipients, which was accompanied to increased expression of CD25 on CD4$^+$ and CD8$^+$ Tcon cells in SOC recipients (p=0.02). In perfusates of OCS lungs, significantly higher pro-inflammatory cytokine levels were detected compared to perfusates of SOC lungs, especially the antagonist IL-1RA. Remarkably, in these OCS perfusates, FoxP3 expression of CD4$^+$CD25$^+$ T cells was maintained but significantly decreased in SOC perfusates (p<0.001). **Conclusions**: Preservation using the OCS has a strong immunological impact towards an anti-inflammatory milieu systemically and maintenance of FoxP3 expression in Tregs in BAL reflecting human data of the INSPIRE trial.

P.C3.03.04

A unique CD56^{dim} NK cell subset, which is also present in lung perfusates, increases in the periphery of lung transplant recipients

R. Bellmàs Sanz[1], *M. Seyda*[1], *B. Wiegmann*[2], *K. Bläsing*[1], *C. Neudörfl*[1], *A. Knöfel*[2], *I. Tudorache*[2], *C. Kühn*[2], *M. Avsar*[2], *A. Haverich*[2], *G. Warnecke*[2], *C. S. Falk*[1];
[1]*Institute of Transplant Immunology, Hannover Medical School, Hannover, Germany,* [2]*Department of Cardiothoracic, Vascular and Transplantation Surgery, Hannover Medical School, Hannover, Germany.*

Purpose: Biomarkers to predict short and long term outcome after lung transplantation are urgently needed. Monitoring of the longitudinal dynamics lymphocyte subsets after lung transplantation represents a feasible strategy for the identification of potential biomarkers for rejection. In this study we defined the phenotype and kinetics of NK and T cell subsets in recipient blood and compared them with their counterparts in perfusates. **Methods**: Perfusion solutions and blood were obtained before (pre Tx), directly after (T0), 24h (T24) and 3 weeks post transplantation from 60 lung transplant recipients. T and NK lymphocyte subsets from peripheral blood and perfusates were analyzed by multicolor flow cytometry. **Results**: At T0, NK cells increased in recipient blood compared to pre Tx values (p=0.01) and declined at T24 (p=0.04), whereas T cells decreased at T0 (p=0.005) and recovered at T24. The phenotype of these NK cells consisted of CD56^{dim} CD16$^+$ CD161$^+$ cells, with high KIR$^+$ NK cell proportions and significantly elevated CD69 and CD25 expression. T cells at T0 were also enriched for CD69$^+$, CD25$^+$ and KIR$^+$ subsets. This was the prevalent T and NK cell phenotype in perfusates, a unique compartment containing T and NK cell subsets significantly different from matched PBMCs. **Conclusion**: After lung transplantation, CD56^{dim} NK cells with a unique phenotype increase in recipient blood, which raises the question whether NK cells are recruited rapidly into the periphery and/or donor cells are migrating out of the transplanted lung. The similarity of these cells with perfusate cells may indicate their donor origin.

P.C3.03.05

Multiplex KIR and HLA class I genotyping using Next Generation Sequencing

L. Closa[1,2], *F. Vidal*[3,2,4], *M. J. Herrero*[1], *J. L. Caro*[1,2];
[1]*Immunogenetics and Histocompatibility Laboratory, Blood and Tissue Bank, Barcelona, Spain,* [2]*Transfusional Medicine Group, Vall d'Hebron Research Institute, Autonomous University of Barcelona (VHIR-UAB), Barcelona, Spain,* [3]*Congenital Coagulopathy Laboratory, Blood and Tissue Bank, Barcelona, Spain,* [4]*CIBER of Cardiovascular Diseases (CIBERCV), Madrid, Spain.*

The killer cell immunoglobulin-like receptors (KIR) are considered the most polymorphic Natural Killer (NK) cell regulators, binding HLA class-I molecules or still unknown ligands. Lately, the interest on KIR genotyping has increased as it has been shown their importance in the identification of the best possible donors for Hematopoietic Stem Cell Transplantation (HSCT) to obtain graft-versus-leukemia effect.
Currently, routine protocols to determine gene content of KIR cluster are exclusively performed by PCR-SSO and PCR-SSP. To improve the study of these genes, we developed a multiplex Long-Range PCR strategy suitable for simultaneous high-resolution HLA class I and KIR genotyping by Next Generation Sequencing (NGS). This protocol allows the amplification of 17 KIR genes and pseudogenes and HLA class I with further sequencing using an Illumina sequencer. The bioinformatics analysis for KIR genotyping was performed using in-house gene-specific virtual probes by CLC Genomics Workbench 11 and the HLA genotyping by GenDx NGSengine software 2.8.0. To validate the method reliability, 96 previously characterized genomic DNA samples were used.
When specific KIR was present, an average of 415 gene-specific virtual probes was detected, meanwhile when it was absent, the average was 6, facilitating the cutoff establishment. Also, the rate of concordance for both KIR and HLA was 100% compared with previous results.
In conclusion, we demonstrated that the multiplex PCR NGS-based strategy could provide a much more efficient and economic method for KIR-ligand genotyping at presence-absence level compared to current techniques. Moreover, reach the allelic level will be possible when specific software becomes available.

P.C3.03.06

Immunosuppressive drug withdrawal late after liver transplantation leads to an improvement of lipid metabolism, a reduction of infections and an increase in gamma-delta Vd1 perforin and granzyme B positive T-cells

A. A. Duizendstra[1], *R. J. de Knegt*[1], *M. G. Betjes*[2], *N. H. Litjens*[2], *J. Kwekkeboom*[1];
[1]*Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, Netherlands,* [2]*Department of Internal Medicine - Division of Nephrology and Transplantation, Erasmus University Medical Center, Rotterdam, Netherlands.*

Background: Liver transplant (LTx) recipients need lifelong treatment with immunosuppressive drugs (IS) to prevent rejection. Long-term use of IS is accompanied by adverse effects. Occasionally LTx recipients can be fully withdrawn from IS and are considered to be tolerant towards their graft. Studies indicate that the Vδ1/Vδ2 ratio of γδT-cells is a useful biomarker to identify tolerant LTx recipients.
Objective: To assess clinical and immunological effects of IS withdrawal late after LTx.
Methods: LTx recipients withdrawn from IS (TOL; n=13), and a control group on IS (CTRL; n=22) matched for time after LTx, age, gender and CMV serostatus were included. Liver and kidney function, lipid profile and infections before and after withdrawal or matching time points were evaluated. PBMCs were characterized by flow-cytometry.
Results: Liver function (bilirubin, AST, ALT) levels significantly improved in TOL after IS withdrawal, whereas kidney function did not. LDL levels and total number of infections significantly decreased in TOL after IS withdrawal, but not in CTRL. No differences were found in Vδ1/Vδ2 ratios between both groups, but Vδ1+GranzymeB+Perforin+ γδT-cells were significantly increased in TOL compared to CTRL.
Conclusion: After IS withdrawal no deterioration of graft function is observed, lipid metabolism improves and total number of infections decrease, but kidney function does not improve. It is likely that kidney damage in LTx recipients is irreversible after long-term IS therapy. Vδ1/Vδ2 ratio is not increased in TOL, which may be due to matched CMV serostatus of CTRL, but in TOL enhanced cytotoxic status of Vδ1 γδT-cells is observed.

P.C3.03.07

Alloantibody can detect a single aminoacid change at position 59 in HLA-B27

N. Egri Córdoba, *M. Digón Doral, A. Manchón Castillo, E. Palou Rivera, J. Martorell Pons;*
Department of Immunology, Hospital Clínic of Barcelona, Spain.

Abstract
Background: HLA antibodies identification by SAB evidences diversity of alloantibody reactivity between alleles with a common first field but different second field (e.g. B*27:03;B*27:05;B*27:08) corresponding to different proteins of the same "serological specificity".
Objectives: Quantify and analyze the differences in reactivity of different HLA-B*27 proteins with common first field but different second field.
Methods: Sera from 1.100 patients on kidney transplant waiting list were analyzed with KIT Lifecodes LSA Immucor. 304 had anti HLA-I. Those positive with at least one B*27 protein were selected. Patterns had been quantified and their rationale was analyzed at the eplet and sequence level.
Results: We found 66 patients with some reactivity with B*27 alleles. Surprisingly, 14/66 (22%) had the pattern (B*27:03-,B*27:05+,B*27:08+) not explained by described eplets. Allele B*27:03 has a single discrepancy with B*27:05&B*27:08 in position 59 Histidine/Tyrosine that should explain this pattern. The rest of the patterns are explained by several eplets as (B*27:03+,B*27:05+,B*27:08+) explained by verified 65QIA, 69AA, 80TLR, 82LR, 131S, 163EW or non verified 66IC, 71KA, 69AT, 76ED, 102DV, 156LA, 170RY, 193PI (37/66); (B*27:03-, B*27:05-,B*27:08+) explained by 76ESN (7/66); (B*27:03+,B*27:05+,B*27:08-) explained by 80TLR, 82LR (7/66). Probably p59H can modify reactivity with eplets 65QIA, 69AA and 163EW that are in a short distance in tridimensional structure.
Conclusion: A single change at position 59 Histidine/Tyrosine can explain differences in alloantibody reactivity in 22% of B*27 reacting patients. This change is not involved in described eplets.

P.C3.03.08

Corneal infiltrating lymphocytes in corneal rejection: pilot study

F. Esen[1,2], *E. Cetin*[2], *S. Genc*[3], *G. Deniz*[2], *M. Taskapili*[3], *H. Oguz*[1];
[1]*Istanbul Medeniyet University School of Medicine, Department of Ophthalmology, Istanbul, Turkey,* [2]*Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Istanbul, Turkey,* [3]*University of Health Sciences, Beyoglu Eye Research and Training Hospital, Istanbul, Turkey.*

Corneal transplantation is the most common transplantation procedure globally and corneal rejection is still an important complication limiting its success. The aim of this study was to define corneal infiltrating lymphocytes in patients with corneal rejection.
Two patients who needed graft exchange due to corneal rejection were included into the study. Corneal infiltrating lymphocytes were isolated with tumor dissociation kit with gentle MACS Dissociator and were analyzed by flow cytometry. The sample of the first patient was stained with anti-human CD45-FITC/CD14PE, anti-human CD3-FITC/CD19-PE and the second sample was stained with CD45-FITC/CD14-PE, anti-human CD3-FITC/CD4-PE monoclonal antibodies.
The first case was a 36 years-old, male, keratoconus patient who had keratoplasty 8 years ago and the second case was a 23 years-old, male, keratoconus patient who had keratoplasty 3 years ago. Both patients needed rekeratoplasty due to corneal rejection and opacification, which was unresponsive to medical treatment. The flow cytometry analysis revealed that 33% of the infiltrating cells were $CD3^+$ T and 17% were $CD19^+$ B lymphocytes. Among the $CD3^+$ T lymphocytes, expression of CD4 was 19.7%.
This pilot study demonstrated that corneal infiltrating lymphocytes could be isolated from human corneal tissue with kits designed to extract lymphocytes from other solid tissues. Previous animal model work and human aqueous humor studies highlighted role of T cell and B cell cooperation in corneal rejection. This pilot study demonstrated for the first time human corneal infiltration by these cells. Further work on this topic would contribute better understanding of the immunology of human corneal rejection.

P.C3.03.09

PIRCHE-II is related to graft failure after kidney transplantation

K. Geneugelijk[1], *M. Niemann*[2], *J. Drylewicz*[1], *H. G. Otten*[1], *E. Spierings*[1], *on behalf of the PROCARE consortium;*
[1]*Laboratory of Translational Immunology, UMC Utrecht, Utrecht, Netherlands,* [2]*PIRCHE AG, Berlin, Germany.*

Individual HLA mismatches may differentially impact graft survival after kidney transplantation. Therefore, there is a need for a reliable tool to define permissible HLA mismatches in kidney transplantation. We previously demonstrated that donor-derived Predicted Indirectly ReCognizable HLA Epitopes presented by recipient HLA class II (PIRCHE-II) play a role in *de novo* DSA formation after kidney transplantation. In the present Dutch multi-center study we evaluated the possible association between PIRCHE-II and kidney graft failure in 2,918 donor-recipient couples that were transplanted between 1995 and 2005. For these donors-recipients couples, PIRCHE-II numbers were related to graft survival in univariate and multivariable analyses. Adjusted for confounders, the natural logarithm of PIRCHE-II was associated with a higher risk for graft failure (HR:1.13, 95% CI:1.04-1.23, p=0.003). When analyzing a subgroup of patients who had their first transplantation, the hazard ratio of graft failure for ln(PIRCHE-II) was higher compared to the overall cohort (HR:1.22, 95% CI:1.10-1.34, p<0.001). PIRCHE-II demonstrated both early and late effects on graft failure in this subgroup. These data suggest that the PIRCHE-II may impact graft survival after kidney transplantation. Inclusion of PIRCHE-II in donor-selection criteria may eventually lead to an improved kidney graft survival.

P.C3.03.10

Manipulation of macrophage phenotype by genetic, pharmacologic and magnetic interference

M. Kloc[1], *J. Kubiak*[2], *W. Chen*[1], *J. Wosik*[3], *R. M. Ghobrial*[1];
[1]*The Houston Methodist Hospital, Houston, United States,* [2]*Unversity of Rennes, Rennes, France,* [3]*University of Houston, Houston, United States.*

In all eukaryotic cells the cell shapes and the cell functions are reciprocally related. Thus, the genetically, biochemically or mechanically/magnetically enforced change in the cell shape will profoundly reverberate at the cell functions. Cell shape depends on the actin filament cytoskeleton, which is regulated by the small GTPase RhoA and its downstream effector ROCK kinase. We studied how genetic or pharmacologic interference with RhoA pathway changes phenotype, shape and functions of immune cells such as macrophages. We found that RhoA-deletetion or RhoA/ROCK inhibition elongates macrophages and disrupts their actin-dependent organelles and functions such as Golgi complex and receptor recycling pathway. All these changes block macrophage ability to mount post-transplantation immune response in the rodent transplantation model system. We also found that the macrophages exposed to the magnetic field acquire phenotype that mimics the RhoA-interference phenotype. These findings have a potential to be used in development of novel anti-rejection therapies in clinical organ transplantation and anti-cancer and anti-metastatic therapies.

P.C3.03.11

The relationship between the C1q-binding ability and the IgG subclass pattern of anti-HLA antibodies

A. Navas[1], *J. Molina*[2,1], *M. Agüera*[3,1], *A. Jurado*[2,1], *A. Rodríguez-Benot*[3,1], *P. Aljama*[3,1], *C. Alonso*[2,1], *R. Solana*[1];
[1]*Maimonides Biomedical Research Institute of Cordoba (IMIBIC)/ Reina Sofia University Hospital/ University of Cordoba, Cordoba, Spain,* [2]*Department of Allergy and Immunology, Reina Sofia University Hospital, Cordoba, Spain,* [3]*Department of Nephrology, Reina Sofia University Hospital, Cordoba, Spain.*

The presence of antibodies against donor HLA antigens (DSA) is the main barrier prior to transplantation. However, not all DSA seem to involve the same risk for allograft failure. Several modifications of the standardized single antigen bead (SAB) assay have been introduced to identify properties of anti-HLA antibodies which could determine their pathogenic potential. The aim of this study was to ascertain the relationship between the complement-binding ability of anti-HLA antibodies and their IgG subclass profile.
We studied 10 serum samples belonging to highly-sensitized patients awaiting single-kidney transplantation at Reina Sofia University Hospital using the modified SAB-C1q and SAB-subclass assays.
Among the 1,346 Luminex-beads analyzed, 248 (18.4%) were able to bind complement. The presence of IgG1 and/or IgG3 subclasses were found in 274 (25%) non-C1q-binding and 236 (95.2%) C1q-binding anti-HLA antibodies. The correlation per bead between the MFI value by SAB-C1q assay and the MFI value of each subclass was of R_{IgG1}=0.492, R_{IgG2}=0.184, R_{IgG3}=0.376 and R_{IgG4}=0.136. When analyzing the C1q-binding ability according to the IgG subclass pattern, we found that the MFI value in SAB-C1q assay of antibodies comprised of a mixture of subclasses (IgG1+IgG2+IgG3/4) was significantly higher than that of those comprised of isolated IgG1 (p<0.001) and of IgG1+IgG2 (p<0.001).
Both C1q-binding and non-C1q-binding anti-HLA antibodies may be comprised of strong complement-binding subclasses (IgG1/IgG3). IgG1 is the subclass which best correlates with the ability to bind C1q. Antibodies comprised of a mixture of subclasses (IgG1+IgG2+IgG3/4) are more likely be able to bind C1q.

P.C3.03.12

Alternative to CDC Crossmatch in patients candidates to renal transplantation treated with Rituximab

P. Lapuente Suanzes[1], *T. Mateu Albero*[2], ***I. Nieto Gañán***[1], *J. Castañer Alabau*[1];
[1]*Immunology Department Hospital Universitario Ramón y Cajal, Madrid, Spain,* [2]*Immunology Department Hospital Universitario la Princesa, Madrid, Spain.*

Introduction: Rituximab (anti-CD20) can modify the result of crossmatch (XM) in renal transplantation, being necessary to look for complementary studies that allow to interpret the result of XMs only based on the presence or absence of anti-HLA antibodies.
Case presentation: A 62-year-old man with rheumatoid arthritis treated with Rituximab. He required a kidney transplant for thrombosis of the left renal artery and loss of function. Despite the absence of anti-HLA antibodies, both XMs by complement-dependent cytotoxicity (XM-CDC) and by flow cytometry (XM-FC) were positive, the latter being only positive for B cells. An assay was designed to confirm that positivity was due to the presence of Rituximab in serum.

Material and methods: XM-FC was performed by adding anti-CD20 monoclonal antibody (mAb) to the usual protocol and comparing the differences between incubating donor cells with patient's serum before or after labelling with anti-CD20 mAb. BD FACSCanto-II flow cytometer and FACSDiva software were used to acquire and analyse the samples.
Results: XM-FC was negative for B cells pre-incubated with anti-CD20. In addition, the CD20 IMF was higher in comparison with the B cells previously incubated with the patient's serum, being in both cases clearly lower than controls.
Conclusions: This trial allows us to demonstrate that the positivity in XM-FC of these patients is due to the action of Rituximab. Furthermore, knowing the fact that Rituximab does not contraindicate the transplant, the correct interpretation of the XMS made to these patients has a crucial clinical importance since that kidney transplant depends on it.

P.C3.03.13
In search of renal transplantation options for hyperimmunized patients

I. Nieto Gañán[1], *S. Elías Triviño*[2], *S. Jiménez Álvaro*[3], *C. Galeano Álvarez*[2], *A. Collado Alsina*[4], *M. Sacén*[5], *A. Fernández Rodríguez*[2], *J. Castañer Alabau*[1];
[1]Immunology Department Hospital Universitario Ramón y Cajal, Madrid, Spain, [2]Nephrology Department. Hospital Universitario Ramón y Cajal, Madrid, Spain, [3]Nephrology Department Hospital Universitario Ramón y Cajal, Madrid, Spain, [4]Instituto Ramón y Cajal de Investigación Sanitaria, Madrid, Spain, [5]Nephrology Department. Hospital de la Raza, Ciudad de Mexico, Mexico.

Introduction: One essential problem in the waiting lists for kidney transplantation are hyperimmunized patients. The high sensitivity of solid-phase techniques for the study of antibodies and definition of virtual crossmatch make transplant difficult in them. Therefore, it should be considered the possibility of receiving grafts looking for assumable risks depending of fluorescence intensity values of DSAs.
Material and methods: We followed 41 patients with a mean of 47.48 months in waiting list and average panel reactive antibody (PRA) of 52.4% transplanted 2014-2017 with negative CDC-XM and positive virtual-XM (maximun allowed 1 DSA>100.000SFI+1 DSA>40.000 SFI). Correlations were sought between the evolution of the graft based on clinical criteria and immunological studies (anti-HLA antibodies by Luminex and Eplets compatibility by HLA-Matchmaker).
Results: 6 patients lost the graft (PRA≥90%). 3 patients presented humoral rejection, two of them with graft loss (4.9%). A correlation was observed between the number of incompatible donor-recipient eplets and the anti-HLA antibodies titre (R^2=0.37). Patients who received plasma exchange posttransplantation had a decrease in the DSAs titre (except one, with no graft loss).
Conclusions: Currently a large number of patients included in kidney transplant lists are hyperimmunized, being necessary to look for transplant protocols for them with reduced possibility of humoral rejection. Our series seems to indicate that renal transplantation with DSAs imply an assumable risk. However, studies with larger series of patients and with a longer follow-up period are needed to stablish threshold titres of anti-HLA antibodies. Most hyperimmunized patients (PRA>95%) could receive personalized immunosuppressive protocols post-transplant.

P.C3.03.14
Detection of a new HLA-A*30 allele in a donor from the NMDP register

M. Vilches-Moreno, ***M. San Jose-Cascon****, E. Garcia-Moreno, A. Nieto;*
UGC Hematology, Immunology and Genetics, Hospital Universitario Puerta del Mar, Cadiz, Spain.

Introduction: High resolution typing for HLA molecules is a crucial component in donor unrelated stem-cell transplantation. Mismatches between patient and donor may lead to transplantation-related complications resulting in morbidity or mortality. Here we describe the identification of a novel HLA-A*30 null allele in the course of high resolution confirmatory typing that precluded donation from an *a priori* matched unrelated bone marrow donor.
Materials and Methods: HLA alleles were sequenced through exons 2-4 in both directions using reagent kit AlleleSEQR HLA and analysed with Assign SBT software. Primers were designed to separately amplificate and sequence the third exon of HLA-A*30 and HLA-A*01. A PCR-SSP strategy was designed to further confirm the presence of the new allele.
Results: Typing of the sample by registry was A*01:UAUY, A*30:PXPW. The sequence obtained using HLA-A AlleleSeqr kit did not fully match with any allele pairs in the IMGT/HLA and showed two heterozygous mismatches with respect to HLA-A*01:01/*30:01 allele pairs. Specifically, codon 175 showed G/**T** at position 3 and codon 176 showed A/**T** at position 1 (mismatches in bold type). The phase of these changes was ascertained both by sequencing exon 3 of A*01 and A*30 separately from each other and by in house designed PCR-SSP. Both approaches showed that the mismatched TT were in the A*30 allele. The change in codon 176 generates a stop codon (TAG) in the A*30 allele most probably abolishing its surface expression.
Conclusions: We have found a probably null HLA-A*30 allele with sequence variations not described so far.

P.C3.03.15
Prevalence and impact of preformed and de novo anti-HLA donor specific antibodies in liver transplantation

M. Papachristou[1], *A. Fylaktou*[1], *M. Daoudaki*[2], *E. Cholongitas*[3], *T. Karampatakis*[1], *A. Anastasiou*[1], ***A. Sarantopoulos***[4], *G. Chatzika*[1], *L. Vagiotas*[5], *I. Fouzas*[5];
[1]National Peripheral Histocompatibility Center-Immunology Department, Hippokration General Hospital, Thessaloniki, Greece, [2]Biochemistry Laboratory, Aristotle University of Thessaloniki, Medical School, Thessaloniki, Greece, [3]First Department of Internal Medicine, Medical School of National & Kapodistrian University of Athens, Athens, Greece, [4]2nd Department of Internal Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece, [5]Solid Organ Transplantation Center, Aristotle University of Thessaloniki Medical School, Hippokration General Hospital, Thessaloniki, Greece.

Introduction: The prevalence and impact of pre-existing and de novo anti-HLA donor-specific antibodies (DSAs) after orthotopic liver transplantation (OLT) is still controversial. We investigated the role of preformed DSAs in the antibody-mediated rejection (AMR) and risk factors implicated in the emergence of DSAs and allograft dysfunction after OLT.
Material: A total of 65 liver transplant patients were tested for anti-HLA antibodies, with single antigen bead technology (SAB), before, 1, 3, 6 and 12 months after transplantation, and thereafter annually, along with other risk factors. **Results:** Sixteen out of 65 patients (24.6%) had circulating pre-existing anti-HLA antibodies and 4 of them (25%) had DSAs. All patients positive for anti-HLA antibodies (100%) presented allograft dysfunction. Fourteen out of 65 patients (21.5%) had circulating de novo DSAs and 12 out of 14 (85.7%) presented allograft dysfunction. The investigated risk factors were: recipient and donor age, time at waiting list, cold ischemia time (CIT), cytomegalovirus (CMV) infection, immunosuppression regimen, de novo DSAs, model for end-stage liver disease (MELD), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), direct bilirubin (DBIL) and total bilirubin (TBIL) peak post-transplant and alkaline phosphatase (ALP). The multivariate analysis showed that de novo DSAs and time at waiting list were independent risk factors for allograft dysfunction.**Conclusion:** This is one of the few reports where the presence of DSAs is associated with the clinical outcome of an OLT. Further research may confirm the clinical value of these antibodies in developing assessment algorithms of pre-operational candidates and post-operational patients.

P.C3.03.16
Analysis of monocyte derived macrophages from lung transplantation patients

I. Schreurs[1], *B. Meek*[1], *C. van Moorsel*[1], *H. D. Luijk*[2], *J. M. Kwakkel-van Erp*[2], *E. Oudijk*[1,2], *D. van Kessel*[1], *J. C. Grutters*[1,2];
[1]Sint Antonius Hospital, Nieuwegein, Netherlands, [2]UMC Utrecht, Utrecht, Netherlands.

Lung transplantation (LTx) is a last treatment option for patients with an end-stage pulmonary disease. Standard immunosuppressive medication is mainly focussed on the acquired immune system. How this medication affects the monocyte-macrophage lineage is not known. The goal of this study is to determine how monocyte subsets and differentiation towards macrophages is affected in LTx patients. Fresh whole blood samples were analysed and monocyte subsets quantified using flow cytometry. To obtain monocyte-derived macrophages, PBMCs were collected from LTx patients and matched controls. Monocytes were differentiated and macrophage phenotype and cytokine production were analysed. Total peripheral monocyte numbers were decreased in LTx patients compared to healthy controls. The ratio between monocyte subsets showed a shift with increased classical monocytes and decreased non-classical monocytes. Surface marker expression levels of TLR2, CD163 and CD36 were increased and CD86 was decreased. Experiments analysing phenotype and cytokine production in macrophages are currently in progress. The differences found in monocyte count and expression of surface markers are thought to indicate a shift towards an M2 macrophage in LTx patients. In vitro confirmation is ongoing.

P.C3.03.17
The function of external respirationin patients after kidney transplantationin condition of immunosuppressive therapy

O. Shtepa*, O. Kuryata;*
SE "Dnipripetrovsk medical academy of Health Ministry of Ukraine", Dnipro, Ukraine.

The aim of our study was to evaluate the changes of the function of external respiration (FER) in patients after kidney transplantation in condition of immunosuppressive therapy and to assess the relationship between the level of cyclosporin A and tacrolimus with FER indicators. Materials and Methods. The study included 37 patients after kidney transplantation. The first group included 27 patients who under the immunosuppressive therapy regimen received cyclosporine at an average dose of 225 [175-350]mg/day, the second group included 10 patients who received tacrolimus at an average dose of 8.25 [5.0- 9.0]mg/day. Control group - 12 healthy persons. Cyclosporin A and tacrolimus were assayed with electrochemiluminescence immunoassay analyzer in addition to general examination and spirometry.
Results. Our data indicated the presence of significant difference ($p<0.05$) in patients of the first and second groups between the indicators of the VC_{max} (78[71-90]% and 76.5[72-78]%), FVC (93[85-99]% and 95[91-98]%), PEF (82[64-94]% and 80[69-84]%), MEF_{25-75} (75[66-112]% and 82.5[67-90]%) relative to the FER of the control group: VC_{max} (102.5[98-113]%), FVC (107.5[105.5-124]%), PEF (99.5[95-102.5]%), MEF_{25-75} (98.5[97.5-101.5]%).

In both groups, a statistically significant negative correlation was found between the indicators of the VC_{max}, FVC and the level of cyclosporin A (R=-0.69, p<0.0001 and R= -0.4, p<0.037) in the first group and FVC and tacrolimus (R=-0.72, p<0.018) in the second group.
Conclusions. A moderate decrease in the VC_{max} values in patients after kidney transplantation in condition of immunosuppressive therapy requires monitoring FER and conducting such patients by nephrologists together with specialists in the pulmonological profile.

P.C3.03.18
The role of sphingosine-1-phosphate in vascular permeability

G. C. Wilkins[1], *S. Ali*[1], *N. S. Sheerin*[1,2], *J. A. Kirby*[1];
[1]*Newcastle University, Newcastle upon Tyne, United Kingdom,* [2]*Freeman Hospital, Newcastle upon Tyne, United Kingdom.*

Introduction
Organ transplantation is the preferred treatment for end-stage organ failure. However, to meet the ever increasing demand for donor organs, marginal organs are more frequently accepted. Organ quality can be improved by *ex vivo* perfusion, which also allows for the directed delivery of therapeutics. The signalling lipid sphingosine-1-phosphate (S1P) binds G protein coupled receptors (S1PR1-5) to affect the endothelial barrier. This study was designed to determine the potential of perfusion with S1PR agonists/antagonists to enhance the endothelial barrier, thereby reducing organ oedema and leukocyte infiltration.
Materials and Methods
Human microvascular endothelial cells (HMEC-1) were treated with either vascular endothelial growth factor (VEGF) or H_2O_2 and their effects on S1P receptor gene expression measured using qPCR. Concurrently, concentrations of S1P were compared between serum and kidney perfusate using an enzyme-linked immunosorbent assay (ELISA) (n=7). Lastly, HMEC-1 grown on transwells were treated with S1PR agonists and the translocation of Evan's Blue dye used to measure endothelial permeability.
Results
VEGF significantly increased S1PR1 expression ($p \leq 0.05$) and H_2O_2, mimicking reperfusion injury, significantly decreased expression ($p \leq 0.05$). The ELISA showed that concentrations of S1P in serum were significantly higher than in kidney perfusate ($p \leq 0.05$). Treatment with an S1PR1 agonist significantly decreased dye permeability of HMEC-1 ($p \leq 0.05$), whereas treatment with a S1PR3 agonist increased permeability.
Conclusions
This study identifies potential mediators of S1PR gene expression on the endothelium during organ transplantation. As S1P concentrations in perfusate are lower than physiological levels, addition of S1PR1 agonists to the perfusate could enhance the endothelial barrier in transplanted organs.

P.C3.03.19
IMMUNE CROSSMATCH FOR KIDNEY TRANSPLANTATION - IS THERE SPACE FOR METHOD IMPROVEMENT?

M. Zielinski[1], *G. Moszkowska*[1], *H. Zielinska*[1], *A. Dukat-Mazurek*[1], *J. Dębska-Zielkowska*[1], *J. Sakowska*[1], *B. Rutkowski*[2], *A. Dębska-Ślizień*[2], *P. Trzonkowski*[1];
[1]*Department of Clinical Immunology and Transplantology, Medical University of Gdansk, Gdansk, Poland,* [2]*Department of Nephrology, Transplantology and Internal Diseases, Medical University of Gdańsk, Gdansk, Poland.*

There are different laboratory methods for donor-recipient immunological matching before kidney transplantation. Although complement dependent crossmatch is still a gold standard other method have been commonly used like virtual crossmatch (V-XM), and flow cytometry crossmatch (FC-XM). The methods differ in sensitivity and specificity, that may result in dissimilar test results and confusing data interpretation. Recently, cytolytic flow cytometry crossmatch (cytolytic FC-XM) was developed, to detect lytic alloantibodies with a sensitivity of flow cytometry assay. Thus, the aim of this study was to compare the laboratory crossmatch outcome with use of different assays. This was a virtual immunological matching of deceased donors with hypothetical recipients. Serum from 22 sensitized patients was crossmatched with surrogate donors and in all cases V-XM was positive at minimum 5000 MFI cut off. The positive CDC-XM result was noted in 41% of patients, while positive FC-XM in 86% and lytic antibodies (cytolytic FC-XM) were confirmed in 27%. There was a moderate correlation for the CDC reaction and cytolytic FC-XM level, both for total/ B cell enriched lymphocytes pool (CDC-XM) and CD3/CD19 lymphocytes (cytolytic FC-XM). When cut-off value of 7000 MFI of highest DSA was used all the positive CDC-XM cases were identified. Similarly, positive FC-XM was followed by 2500 MFI cut-off value. Our results suggest that donor-recipient immunological matching for kidney transplantation requires different methods to verify the importance of alloantibodies. Thus, there is still space for improvement. This is especially important for immunized patients for successful transplantation.

P.C3.03.20
Lymphocytes B as a potential marker of alloantibodies development after kidney transplantation

M. Zielinski[1], *A. Tarasewicz*[2], *H. Zielinska*[1], *M. Jankowska*[2], *G. Mozkowska*[1], *J. Sakowska*[1], *B. Rutkowski*[2], *A. Dębska-Ślizień*[2], *P. Trzonkowski*[1];
[1]*Department of Clinical Immunology and Transplantology, Medical University of Gdańsk, Gdansk, Poland,* [2]*Department of Nephrology, Transplantology and Internal Diseases, Medical University of Gdańsk, Gdansk, Poland.*

Immune diagnostic after kidney transplant is focused on alloantibodies assessment to confirm humoral rejection episodes. Anti-HLA antibodies development is associated with specific B cells differentiation to long-lived memory cells and molecular signature. Thus, it is interesting whether B cells phenotype can be applied as a marker of humoral immunity activation. This may be beneficial for personalized risk stratification. Low-risk kidney transplant recipients (n=53) were followed-up to 24 months after transplantation for alloantibodies development and signs of organ rejection. Every three months anti-HLA antibodies, naïve/memory B lymphocytes as well as CD5+ B cells phenotype were assessed together with Th1/Th2 and BAFF serum levels. For the 34 recipients, alloantibodies were not present after transplantation, while for 19 others DSA/de novo anti HLA were confirmed. The higher rate was observed 20 months after transplantation. Anti-HLA antibodies development was proceeded by increased in BAFF and INFgamma levels, as well as memory B lymphocytes numbers. It was found, that alloantibodies development was correlated with a number of memory B cells, Rs=0,96 (Spearman rank correlation). Lymphocytes B phenotype monitoring after kidney transplantation is useful for alloantibodies development and may serve as an additional marker of humoral immunity activation. These could be also beneficial for individual risk stratification and tailored immunosuppression protocol development after kidney transplantation. Funding: National Centre for Research and Development, Poland (No. STRATEGMED1/233368/1/NCBR/2014). National Science Centre, Poland (No. NN402420738 and NN402 562440).

P.C3.04 MHC, Stem Cell Transplantation and Regulation

P.C3.04.01
CCR4 and CCR8 chemokine receptor expression on highly suppressive regulatory T cells

G. Adigbli;
Transplantation Research Immunology Group, Oxford, United Kingdom.

Introduction: Human regulatory T cells (Tregs) are a promising therapy for the safe control of transplant rejection. The chemokine receptors CCR4 and CCR8 are expressed by subsets of Tregs with unusually high suppressive activity. However, the mechanisms underlying this enhanced suppressive activity are not fully understood and yet to be explored in transplantation. The aim of this study is to characterise the suppressive abilities of $CCR4^+$ and $CCR8^+$ Treg in transplantation and explore the mechanisms underlying them.
Methods: Peripheral blood human Tregs were sorted by expression of CCR4 and CCR8 surface receptors before being stimulated with anti-CD3/anti-CD28 beads for 14 days and analysed by flow cytometry.
Tregs expanded for 14 days with anti-CD3/anti-CD28 beads were sorted by expression of CCR4 and CCR8 surface receptors and used in an *in vitro* suppression assay. Intracellular interleukin (IL)-10, interferon (IFN)-gamma and IL-17 were also measured.
CCR4 and CCR8 expression in the peripheral blood of recipients of kidney/pancreas transplants with sentinel skin flaps was assessed.
Results: Naïve Tregs upregulate CCR4 and CCR8 expression transiently upon stimulation whereas memory Tregs maintain expression long term.
$CCR4^+$ Tregs are more suppressive than $CCR4^-$ Tregs in a chemotaxis-independent manner and produce greater levels of IL-10 and IFN-gamma.
Treg CCR8 expression is increased in the blood of pancreas/skin transplant recipients undergoing episodes of rejection and may potentially serve as an early marker of rejection.
Conclusion: We hypothesise that CCR4 and CCR8 expression identifies a highly suppressive subset of Treg, which may enhance Treg therapy and monitoring of transplant rejection.

P.C3.04.02
Letrazole and Testosterone Combination Stimulates Bone Marrow Mesenchymal Stem Cell Proliferation Without Altering Their Characteristics

B. Aru*, H. Dagdeviren, T. Simsek, G. Yanikkaya Demirel;*
Yeditepe University, Istanbul, Turkey.

Introduction: Due to their immunomodulatory properties which are proven by in vitro studies and clinical trials, mesenchymal stem cells (MSCs) are considered as the most promising cellular therapy agents. However, since they present a limited population in bone marrow specimens and they undergo senescence and lose characteristic features as the passage number increases; it is an important issue to increase their proliferative capacity before re-infusion to patients.Method: Bone marrow mononuclear cells were isolated from 11 individuals' (healthy n:2 patient: 9) samples by eliminating erythrocytes with ammonium chloride lysis solution. Cells were characterized according to their surface markers ($CD73^+CD90^+CD105^+CD34^{neg}DD45^{neg}$) at the 3rd passage.

Two different doses of testosterone or 17-β estradiol (10 nm and 100 nm) and letrazole (1000 nm) with both doses of testosterone were applied for 72 hours. Proliferation rate was evaluated with MTS assay. DNA content and cell surface markers were analysed with Beckman Coulter FC500 flow cytometry.Results: Both MTS assay and DNA cell cycle analysis showed that testosterone, 17-β estradiol and testosterone combined with letrazole supplements in both doses were stimulating cell proliferation without changing expression levels of CD73, CD90, CD105 and CD34, CD45 markers. Conclusion: In this study, we showed that testosterone stimulates proliferation of BM-MSCs when its' aromatisation is inhibited with letrazole addition without any effect on surface markers. Gene level changes with these treatments will be explored in further studies.

P.C3.04.03

The presence of atopic dermatitis in heart-transplanted pediatric patients is associated with an immune Th2 polarisation

*J. Lopez-Abente[1], M. Camino[2], N. Gil[2], E. Panadero[2], M. Campo[2], M. Pion[1], R. Correa-Rocha[1], **E. Bernaldo de Quiros**[1];*
[1]Instituto de Investigacion Sanitaria Gregorio Marañon, Madrid, Spain, [2]Hospital General Universitario Gregorio Marañon, Madrid, Spain.

Introduction: The incidence of comorbidities, such as atopic dermatitis (AD), has increased in pediatric heart transplantation during the last decades. Their treatment remains a significant challenge in transplantation context. Therefore, understanding the immune mechanisms underlying the disease is important to improve the management of this comorbidity. This study assessed whether potential immune alterations associated with regulatory T cells (Treg) and Th1/Th2 imbalance could be related to AD in heart-transplanted children. Materials and Methods: This single-centre, cross-sectional, observational study included 11 AD and 11 non-AD heart-transplanted aged-matched pediatric patients. Peripheral blood samples were obtained, and immune populations were analysed using flow cytometry. Results: We observed that age at transplant was significantly lower in the AD group. In addition, the development of AD after transplant was associated to a higher frequency of IL-4-secreting CD4+ T cells, a decrease in the ratio of IFN-γ to IL-4-secreting CD4+ T cells, an increase in the frequencies of differentiated Treg and eosinophilia. Non-AD patients presented a negative correlation between Treg and IL-4-secreting CD4+ T-cell frequencies, however, such correlation was lost in AD patients. Conclusion: the loss of the correlation between Treg and IL-4-secreting CD4+ T cells suggested the potential incapacity of Treg to prevent the expansion of Th2 cells, that are crucial players in AD development and thus potential therapeutic targets. Funding: This work was supported by grants from Instituto de Salud Carlos III (ISCIII) co-financed by FEDER funds (PI15/00011; ICI14/00282; PI15/00923). J.L-A is supported by an IISGM pre-doctoral grant.

P.C3.04.04

Generation of SLA-silenced porcine pancreatic islets to support graft survival after xenogeneic transplantation

***M. Carvalho-Oliveira**[1], E. Valdivia[1], Y. Yuzefovych[1], O. Pogozhykh[1], R. Schwinzer[2], B. Petersen[3], J. Seissler[4], R. Blasczyk[1], C. Figueiredo[1];*
[1]Institute for Transfusion Medicine, Hannover Medical School, Germany, Hannover, Germany, [2]I2. Transplantation Laboratory, Clinin for General, Visceral and Transplantation-surgery, Hannover, Germany, [3]3. Department of Biotechnology, Institute of Farm Animal Genetics, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Hannover, Germany, [4]4. Diabetes Research Group, Medizinische Klinik IV, Medical Center of the University of Munich, Munich, Germany.

Introduction: The prevalence of diabetes increased over the last decades. Xenotransplantation of porcine pancreatic islets may offer an alternative source to circumvent the limitations posed by the lack of donors, but immune rejection remains a major concern. Here, we have investigated the feasibility to silence SLA class I and II expression to decrease xenogeneic immune responses. Methods: Silencing of SLA class I and II was performed using lentiviral vectors for the delivery of specific short hairpin RNAs (shRNA) targeting β2-microglobulin (β2m) or class II transactivator (CIITA), respectively, into islet cell clusters or monolayers. The lentiviral vectors encoded for GFP or Nanoluciferase as reporter genes. SLA transcripts were evaluated by real-time PCR and protein levels by flow cytometry and fluorescence microscopy analyses. The effect of SLA class I silencing was evaluated in human NK cell cytotoxicity assays. Results: Fluorescence microscopy analyses indicated a successful transduction of the islets in its 3D- structure. Expression of NanoLuciferase was detectable already 24h after transduction. Vector activity remained detectable after two weeks. On islet cells, SLA class I expression was silenced by up to 60.3% and class II by up to 30.4%. Interestingly, silencing SLA class I expression showed a protective effect against NK-cell cytotoxicity. A decrease in NK-cell cytotoxic activity of 55.8% was observed when SLA class I silenced islet-derived cells were used in comparison to SLA class I-expressing cells. Conclusion: These data shows the feasibility to silence SLA expression on porcine islets cells, which may contribute to decrease xenogeneic immune responses after transplantation.

P.C3.04.05

Deciphering anti-HCMV HLA-E-restricted unconventional CD8 T-cell responses in seropositive HCMV⁺ hosts

***B. CHARREAU**[1], N. Jouand[2], C. Bressollette-Bodin[3], N. Gérard[1], M. Giral[4], P. Guerif[5], A. Rodallec[6], R. Oger[7], T. Parrot[7], M. Allard[7], A. Cesbron-Gautier[8], N. Gervois[7];*
[1]CRTI UMR1064, Nantes, France, [2]CRTI UMR1064 and CRCINA UMR1232, Nantes, France, [3]CRTI and Service de Virologie, CHU de Nantes, Nantes, France, [4]CRTI and CHU de Nantes, Nantes, France, [5]CHU de Nantes, Nantes, France, [6]Servide de Virolgie, CHU de Nantes, Nantes, France, [7]CRCINA UMR1232, Nantes, France, [8]EFS Pays de la Loire, Nantes, France.

Human cytomegalovirus (HCMV) causes severe illness and poor outcome in immunocompromised hosts such as transplant recipients and HIV-infected patients. Cytotoxic CD8 T cells against HCMV antigens (pp65, IE1) presented by classical HLA class-I molecules are major cellular components of the protective anti-HCMV immunity. HLA-E-restricted CD8 T cells targeting HCMV UL40 leader peptides (Lp) have been recently reported as unconventional T-cell responses also observed in some hosts but they still need clinical and functional characterization. Our study aimed to provide a qualitative and quantitative *ex vivo* analysis of HLA-E_{UL40} CD8 T-cell responses, in a large cohort (n=144) of kidney transplant recipients and healthy volunteers, and to elucidate determining factors. HLA-E_{UL40} CD8 T-cells were detected in >30% of seropositive HCMV⁺ hosts and may represent >30% of total circulating CD8 αβT cells at a time point. We identified host-related genetic factors (HLA-A*02 and HLA-E genotype) and HCMV strain, determining the sequence of UL40Lp, as critical parameters for this response. HLA-E_{UL40} CD8 are effector memory T cells that appear early post-infection as monoclonal/oligoclonal populations and persist for life. Although specifically induced in response to HCMV infection, a key feature of these cells is their potential ability to be also responsive against self and allogeneic HLA resulting from sequence homology between HLA-ILp and UL40Lp. Thus we established that unconventional HLA-E_{UL40} CD8 T cells belong to the common immune arsenal against HCMV. Their functions remain to be defined toward infection as well as their potential side effect in contexts such as autoimmunity and transplantation.

P.C3.04.06

Association of *PTPN22* +788A allele with kidney rejection episode in western Mexican patients with renal failure that had been transplanted

***L. E. CHAVARRIA BUENROSTRO**[1], J. Macias-Barragan[2], M. Arámbula-Navarro[1], J. F. Topete-Reyes[3], J. F. Muñoz-Valle[1], M. G. Ramirez-Dueñas[1], P. E. Sanchez-Hernandez[1], T. Garcia-Iglesias[1], A. L. Pereira-Suarez[1], R. Parra-Michel[3], M. Montoya-Buelna[1];*
[1]UNIVERSIDAD DE GUADALAJARA, GUADALAJARA, Mexico, [2]UNIVERSIDAD DE GUADALAJARA, Ameca, Mexico, [3]INSTITUTO MEXICANO DEL SEGURO SOCIAL, GUADALAJARA, Mexico.

Introduction: kidney transplant allograft rejection episodes have been a major risk factor for adverse transplant outcome, in which regulation of immune cell signaling plays an important role. One of the main proteins involved in immune cell regulation is encoded by *PTPN22* gene. *PTPN22* single nucleotide polymorphisms (SNPs) are associated with autoimmune diseases and transplantation outcome. Studies suggest that rs3399664, +788 G>A *PTPN22* could be related to altered immune response and could be associated with renal graft rejection episode. This study explored the distribution of +788 G>A *PTPN22* polymorphism in Western Mexican general population and examine the association of this SNP with renal graft rejection episode.
Materials and methods: totally 52 patients with renal rejection episodes, 98 renal rejections free episode patients, and 150 healthy subjects were selected. The samples were genotyped for the +788 G>A *PTPN22* gene by PCR-RFLP.
Results: allelic frequencies of +788 G>A *PTPN22* polymorphism for G and A alleles were 98.1%, and 1.9%, respectively; showing a low frequency of A polymorphic allele in a western Mexican population. We found a significant association between patients carrying the heterozygous genotype of +788 G>A *PTPN22* polymorphism and a higher risk for graft rejection episodes as compared to the healthy subjects (p=0.035; OR=4.9, 95% CI=1.26-18.9). The frequency of +788A risk allele was significantly increased in graft rejection episode group patients as compared to the healthy subjects group (p=0.037; OR=4.7, 95% CI=1.24-17.82).
Conclusions: our results show that rs3399664 may confer susceptibility to renal graft rejection episodes development in patients from western Mexico.

P.C3.04.07

***Ex-vivo* expansion of Tregs from Belatacept-Treated Renal Transplant Patients enhances and maintains their suppressive function under inflammatory conditions**

***A. Cortés Hernández**[1], E. Alvarez Salazar[1], J. Alberú Gómez[2], N. Linares Escobar[1], D. Villareal Martínez[1], S. Arteaga Cruz[1], M. Solís Gamboa[3], G. Soldevila Melgarejo[1];*
[1]Instituto de Investigaciones Biomédicas, Mexico, Mexico, [2]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico, Mexico, [3]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán,, Mexico, Mexico.

Immunotherapy with Regulatory T cells (Tregs) is a successful therapy to prevent transplant rejection in experimental models. In addition, some studies have addressed the potential effects of immunosuppressive therapy on Tregs from transplanted patients. In this regard, we reported that Tregs from long-term Belatacept-treated kidney transplant patients displayed a significant reduction in their FOXP3 expression and suppressive capacity but remains unknown whether it is feasible *ex vivo* expand their Tregs for use in immunotherapy. In this study, purified Tregs from long-term Belatacept-treated renal transplant patients were *ex vivo* expanded with polyclonal stimulation in the presence of sirolimus and IL-2. Cell yield after *ex-vivo* expansion was similar between Tregs from Belatacept-treated patients and Tregs from healthy controls. Expanded Tregs from patients expressed high levels of FOXP3, CTLA4, Helios and CCR7 similarly to expanded Tregs from healthy controls.
In addition, expanded Tregs from patients exhibited excellent *in vitro* suppressive function comparable to expanded Tregs from controls.

Most importantly, expanded Tregs from patients maintained their phenotype and suppressive function after *in vitro* stimulation in the presence of the pro-inflammatory cytokines IL-6, TNF-α, IL-4 and IFN-ϒ. In conclusion, $CD4^+CD25^{hi}FOXP3^+$ Tregs from long-term Belatacept-treated patients can be *ex vivo* expanded without loss of their phenotype and function. These data demonstrate that despite the reported alterations of Tregs from transplanted patients maintained long-term with immunosuppressive therapy, it is possible to use Tregs as immunotherapy for induction of allograft tolerance.
Supported by CONACyT #272518. EA, AH and SA were recipients of fellowships from CONACyt.

P.C3.04.08

Implementation of an HLA typing strategy based on Next Generation Sequencing to improve the characterization of umbilical cord blood units

E. Enrich[1,2], *E. Campos*[1], *N. Borràs*[2,3], *L. Mongay*[1], *P. Caro*[1], *C. Lera*[1], *L. Martorell*[3,4], *M. J. Herrero*[1], *J. L. Caro*[1,2], *I. Corrales*[2,3], *S. Querol*[2,4], *F. Vidal*[2,3,5], *F. Rudilla*[1,2];
[1]*Immunogenetics and Histocompatibility Laboratory, Blood and Tissue Bank, Barcelona, Spain,* [2]*Transfusional Medicine Group, Vall d'Hebron Research Institute, Autonomous University of Barcelona (VHIR-UAB), Barcelona, Spain,* [3]*Congenital Coagulopathy Laboratory, Blood and Tissue Bank, Barcelona, Spain,* [4]*Cell Therapy Unit, Blood and Tissue Bank, Barcelona, Spain,* [5]*CIBER of Cardiovascular Diseases (CIBERCV), Madrid, Spain.*

The application of Next Generation Sequencing (NGS) in histocompatibility laboratories has allowed large-scale high-resolution HLA typing, improving and enlarging genotypic information of donors' registries. The objective of this study was to take advantage of an in-house NGS based strategy for *HLA-A*, *-B*, *-C*, *-DRB1* and *-DQB1* typing, to improve HLA characterization of umbilical cord blood units (CBUs) from Barcelona Cord Blood Bank. Specific primers were designed to amplify exons 2, 3 and 4 for HLA class I genes and exons 2 and 3 for class II genes in a single multiplex PCR per patient, using an in-house previously validated procedure. Concurrent sequencing of 384 samples was carried out in Illumina MiSeq 500-cycle runs and NGSengine software was used to HLA genotyping. With this approach 6.000 CBUs and 3.000 samples from cord blood donors' mothers were typed. An average density of 850 Kclusters/mm^2 and 86% of cluster passing filter was obtained; 83% bases had a Q-score $\geq$ 30 and optimal coverage was achieved for the correct typing of each sample. Improvements were performed during its implementation, such us the design of specific primers to identify null alleles or its automation, that allow the typing of up to 768 samples per week. Additionally, 30 new alleles were identified and more than half were submitted to IPD-IMGT/HLA Database. In conclusion, the implementation of this NGS strategy has allowed a cost-effective high-resolution HLA genotyping of a significant fraction of our CBUs repository, which will rebound in a more accurate selection for hematopoietic cell transplantation.

P.C3.04.09

Rare sequence of complications in a pediatric patient after lung transplantation: identification of donor T cells during GvHD, allospecific CTL during acute rejection and CMV-specific CTL after reactivation supports clinical management

C. S. Falk[1], *N. Schwerk*[2], *C. Müller*[2], *I. Tudorache*[3], *C. Neudörfl*[1], *G. Hansen*[2], *A. Haverich*[3], *G. Warnecke*[3];
[1]*MHH, Institute of Transplant Immunology, Hannover, Germany,* [2]*MHH, Department of Pediatric Pneumology, Hannover, Germany,* [3]*MHH, Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover, Germany.*

Background: A 17 year old patient with cystic fibrosis underwent bilateral lung transplantation with immunosuppression (IS) of Tacrolimus, MMF, Prednisone. After 3 months, he developed histology-proven cutaneous GvHD which was successfully controlled by MMF withdrawal but followed by acute rejection of the lung. After treatment by steroid pulse, lung function returned to normal but CMV infection occurred despite valganciclovir prophylaxis. Immunoassays were performed to quantify specific T cell responses as indicator of changes in IS. Methods: Frequencies of HLA-$A32^+$ donor lymphocytes were determined by FACS. ELISpots were performed to detect allospecific and CMV-specific T cells. HLA-A2/NLV-multimer staining was used to detect CMV-specific $CD8^+$ CTL. Results: At the peak of skin GvHD, frequencies of HLA-$A32^+$ donor $CD4^+$,$CD8^+$ T (4%), B (8%) and NK (4%) cells were detected that declined after MMF withdrawal within one week. Simultaneously to improvement of GVHD, acute rejection of the lung developed, accompanied by clonal expansion of A32-allospecific $CD8^+$ CTL, which declined after steroid pulse. HLA-A11-allorestricted, GVHD-mediating CTL were detected at low frequency. With serological detection of CMV, HLA-A2/NLV-specific $CD8^+$ CTL emerged, expanded clonally and persisted for four months. CMV infection disappeared with the appearance of CMV-specific CTL. Conclusions: Using specific immunomonitoring tools, we could follow the clinical course "online" starting with GVHD, followed by rejection and CMV infection. Frequencies of donor lymphocytes, allo- and CMV-specific CTL were indicative for disease stage and adjustment of IS. With full recovery of his lung function, the patient is asymptomatic several months after these complications.

P.C3.04.10

The development of novel multi-parameter flow cytometry panels to identify immune cells in renal transplant recipients undergoing defined exercise program

G. J. Fatania[1,2], *J. E. Pearl*[1], *R. E. Billany*[2,3], *N. C. Bishop*[3], *A. C. Smith*[1,2], *A. M. Cooper*[1];
[1]*Department of Infection, Immunity and Inflammation, Leicester Kidney Lifestyle Team, University of Leicester, Leicester, United Kingdom,* [2]*John Walls Renal Unit, University Hospitals of Leicester NHS Trust, Leicester, United Kingdom,* [3]*School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, United Kingdom.*

Introduction: Renal transplantation improves renal function but do not reduce cardiovascular risk associated with renal disease. Exercise can reduce both conventional and renal-associated cardiovascular risk; however, exercise can positively and negatively affect immune function depending on the nature of the exercise. It is therefore critical to monitor changes in immune cell populations during exercise. To determine the impact of exercise on circulating immune cells of renal transplant recipients we have developed multi-colour flow cytometry panels covering lymphoid and myeloid populations. Methods: A 10-colour lymphocyte panel and two 6-colour myeloid panels (monocytes and dendritic cells) have been designed to assess changes in the frequency of cells within the peripheral blood of renal transplant recipients undergoing defined exercise programmes. Peripheral blood mononuclear cells were isolated and stained with lymphoid lineage markers: CD3, CD4, CD8a, CD19, CD56, and function associated markers: CD45RA, CD45RO, CD127, CD25, and CD197. Monocyte subsets were identified using HLA-DR, CD14, CD16, and their migratory potential determined using CCR2, CCR5 and CX3CR1. Dendritic cell populations were identified using HLA-DR, CD1c, CD11c, CD141, CD123 and CD14. Results/ conclusion: The panels allow reproducible detection of cell populations in thawed peripheral blood cells from healthy volunteers. These panels are now being used to assess immune cell populations in renal transplant recipients undergoing one of three distinct exercise protocols. Changes in cell populations according to exercise will provide crucial information regarding the impact of exercise on immune function. Heart Research UK: RG2650/15/18 to NCB and ACS MR/P011136/1 and WM150032 to AMC

P.C3.04.11

Human adipose-tissue-derived and bone-marrow-derived mesenchymal stem cell-conditioned medium contain immunoregulatory cytokines and exhibit therapeutic potential in wound repair in vitro

S. Gromolak*, M. Paprocka, H. Kraśkiewicz, A. Klimczak;*
Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland.

Introduction Conditioned medium from MSC (MSC-CM) contains mixture of growth and immunoregulatory factors and is perceived as a promising tool for regenerative medicine. We investigated MSC-CMs from two sources: human, immortalized adipose-tissue-derived (ATMSC) and bone-marrow-derived mesenchymal stem cells (BMMSC) for their biological activity for secreted factors and their influence on keratinocyte's proliferation, clonogenicity and migration. **Methods** The expression of biologically active factors produced by ATMSC and BMMSC was evaluated by the C-Series Human-Cytokine-Antibody Array. Keratinocyte cell line (HaCaT) was cultured with different concentration: 12%, 25%, 50%, 100% of MSC-CM. Cell proliferation was assessed by MTT assay, a wound healing assay was used to examine migratory properties of HaCaT, and a colony-forming assay was performed to assess their clonogenic potential. **Results** In both ATMSC-CM and BMMSC-CM 70 biologically active factors were detected out of 120 tested. A number of growth, angiogenic and immunoregulatory factors, including IL-6, IL-8, MCP, GRO and eotaxin were identified. Nine factors were secreted exclusively by BMMSC (e.g. GDNF, SCF) and one cytokine only by ATMSC (IL-7). In functional assays both MSC-CMs stimulated HaCaT proliferation, clonogenic potential and in vitro wound closure compared to control. The optimal effect was observed following treatment with 25% of MSC-CM. **Conclusions** The secretion profile of both lines is similar but not identical. The source of MSC does not significantly influence on MSC-CM's bioactivity and therapeutic potential on wound repair. These data suggest that MSC-CM is a good source of immunomodulatory and trophic factors and may promote tissue repair. Grant NCN No. 2012/07/B/NZ4/018

P.C3.04.12

How tissue niche affect mesenchymal stem cells and how important are their biological properties

U. Kozlowska[1], *K. Futoma*[1], *A. Krawczenko*[1], *D. Patrzalek*[2], ***A. Klimczak***[1];
[1]*Hirszfeld Institute of Immunology and Experimental Therapy PAS, Wroclaw, Poland,* [2]*Medical University, Faculty of Health Science, Department of Physiotherapy, Wroclaw, Poland.*

Introduction: Regenerative potential of human organs is permitted by mesenchymal stem cells (MSC) which reside in various tissues of the body. We investigated biological characteristics of MSC from skin (SK), adipose (AT), skeletal muscle (SM) and bone-marrow (BM) tissues, considering their special features for regenerative medicine. **Materials and Methods**: SK-MSC, AT-MSC, SM-MSC and BM-MSC were isolated and long-term cultured in standard conditions. MSC markers for: CD73, CD105, CD90, proangiogenic CD146, PDGFRα, CD31, and multipotency PW1 were examined by flow-cytometry, and immunofluorecence. The osteogeneic, adipogeneic and chondrogeneic potential by culture in dedicated medium, and 7-day cell fusion after PKH26 and PKH67 labelling were assessed. Cytokine secretion with 27-cytokine multiplex-ELISA screening, and pluripotency (Sox2, Oct4), suppressor (p53) and protooncogenic (c-myc) genes expression with qPCR were analyzed. **Results:** MSC from examined tissues had stable CD90+, CD73+ phenotype, but CD105, CD146 and PDGFR-α expression decreased with subsequent passages.

PW1 was present in SK-MSC, AT-MSC and SM-MSC, but not detectable after P3. Examined MSC were able to triple differentiation, except SM-MSC unable to adipogenesis. ELISA revealed secretion of IL-6, IL-8, MCP-1, VEGF in different concentrations, depending on MSC niche. AT-MSC and BM-MSC were able to fuse with SM-MSC population. Sox2 was stable in SM-MSC and AT-MSC, and SK-MSC showed the highest expression in P1. Oct4, p53 and c-myc expression differs between passages. **Conclusions:** Variability in biological properties of MSC from different niche may influence on MSC quality and therapeutic potential. Proper selection of MSC may determine efficacy of action in regenerative medicine.

P.C3.04.13

PRE-ACTIVATED MESENCHYMAL STROMAL CELLS INDUCE REGULATORY IMMUNE POPULATIONS *IN VIVO* AND PROLONG CORNEAL ALLOGRAFT SURVIVAL

K. Lynch[1], *O. Treacy*[1], *X. Chen*[1], *N. Murphy*[1], *P. Lohan*[1], *G. O'Malley*[1], *S. D. Naicker*[1], *M. D. Griffin*[1], *A. Ryan*[2,1], *T. Ritter*[1];
[1]*Regenerative Medicine Institute (REMEDI), College of Medicine, Nursing & Health Sciences, National University of Ireland, Galway, Galway, Ireland,* [2]*Discipline of Pharmacology, College of Medicine, Nursing & Health Sciences, National University of Ireland, Galway, Galway, Ireland.*

Introduction: Immune rejection is the main cause of corneal allograft failure with at least 30% of transplanted grafts experiencing an immune complication. In this study, transforming growth factor beta 1 treated (TGFβ) mesenchymal stromal cells (TGFβ-MSC) were administered in a murine model of corneal transplantation and rejection free survival (RFS) of corneal grafts was observed over 30 days.
Results: *In vitro,* TGFβ-MSC derived from BALB/c bone marrow were co-cultured with stimulated lymphocytes. TGFβ-MSC significantly suppressed $CD4^+$ and $CD8^+$ lymphocyte proliferation while increasing the frequency of regulatory T cell (Treg) populations.
In vivo, untreated allogeneic control grafts from C57BL/6 mice were uniformly rejected (RFS 20.5±7.75d, n=12) in BALB/c mice. Untreated MSC were not efficacious in prolonging RFS (RFS 18.28±6.71d, n=14). In contrast, corneal allograft rejection was significantly delayed in 70% of TGFβ-MSC treated allograft recipients (RFS 27.23±4.32d, n=13) with significantly lower mean corneal neovascularization and corneal oedema scores.
ex vivo analysis, draining lymph nodes (DLNs), spleens and lungs of both treated and untreated animals were harvested. Significantly increased populations of Tregs were observed in the lungs of TGFβ-MSC treated animals.
Mechanistic Insights, high concentrations of Prostaglandin E2 (PGE2) were detected in TGFβ-MSC/lymphocyte co-culture supernatants. Co-culturing TGFβ-MSC with stimulated lymphocytes in the presence of a PGE2 receptor 4 (EP4) inhibitor returns frequencies of Tregs to control levels.
Conclusion: TGFβ-MSC had an enhanced ability to prolong RFS when compared to untreated MSC in the absence immunosuppressive therapy. This study points toward the use of MSCs as a therapeutic treatment to improve cellular-based therapies.

P.C3.04.14

TNF-α/IL-1β licensed mesenchymal stromal cells promote corneal allograft survival via antigen presenting cell mediated induction of Foxp3+ regulatory T cells in the lung

N. Murphy, *O. Treacy, P. Lohan, K. Lynch, A. Ryan, M. Marcos, M. Griffin, T. Ritter;*
REMEDI, NUIG, Galway, Ireland.

We have previously demonstrated that allogeneic mesenchymal stromal cells (MSCs) administered intravenously (i.v.) pre-transplantation (day-7, day-1) prolong corneal allograft survival, however, syngeneic (recipient-derived) MSCs administered at these timepoints fail to prolong graft survival. Here, we demonstrate *in-vitro* that pre-activation of syngeneic MSCs with pro-inflammatory cytokines TNF-α/IL-1β ($MSC^{TNF\text{-}\alpha/IL\text{-}1\beta}$) enhances MSC's immunomodulatory properties, potently suppressing syngeneic lymphocyte proliferation (10.26%, p≤0.001) compared to untreated MSCs (MSC^{UTR}) (79.68%). Lymphocyte suppression was mediated primarily by up-regulation of MSC's nitric oxide production (NO). *In-vivo*, when administered post transplantation (day+1, day+7) both MSC^{UTR} (50%, p≤0.05) and $MSC^{TNF\text{-}\alpha/IL\text{-}1\beta}$ (70%, p≤0.001) prolong corneal allograft survival compared to allograft controls (0%). The ability of $MSC^{TNF\text{-}\alpha/IL\text{-}1\beta}$ to prolong graft survival was in part mediated by NO production as shRNA knockdown of NO reduced the graft survival rate of $MSC^{TNF\text{-}\alpha/IL\text{-}1\beta}$ treated animals to 25%. MSC-mediated graft survival was associated with increased proportions of regulatory $CD11b^+B220^+$ macrophages and Foxp3+ regulatory T cells (Tregs) in the lung and spleen at day 9 post transplantation while at the time of rejection (day17-19) increased proportions of Tregs were observed in the lung, spleen and crucially the draining lymph nodes of MSC treated allograft recipients. Finally, ex-vivo, we report a mechanism of MSC-mediated Treg induction where MSCs condition CD11b/c+ sorted lung cells to a regulatory phenotype capable of inducing Tregs from naïve lymphocytes. This study highlights the importance of timing and licensing strategies in enhancing MSC therapy and sheds light on how MSCs exert their immunomodulatory properties in the lung via a $CD11b/c^+$ intermediary cell population.

P.C3.04.15

Finding a needle in the haystack; identifying allopeptides in solid organ transplantation

L. Rowntree[1], *P. Illing*[1], *O. Nguyen*[2], *T. Kotsimbos*[3], *A. Purcell*[1], *N. Mifsud*[1];
[1]*Monash University, Melbourne, Australia,* [2]*University of Melbourne, Melbourne, Australia,* [3]*The Alfred Hospital, Melbourne, Australia.*

Transplantation is currently the best therapy for end-stage organ failure, although associated risks of post-transplant morbidity and mortality remain high due to HLA mismatching and common viral infections. Chronic allograft rejection remains a major barrier to overcome, with some evidence that a specific subset of antiviral T cells, which also recognise different HLA allotypes (i.e. cross-reactive), may contribute to allograft rejection. We have identified a CMV-specific HLA-A*02:01-restricted CD8+ T cell with a unique TCR signature (OTN5) that cross-reacts with a defined hierarchy towards HLA-B27 allomorphs (B*27:07>B*27:09>B*27:05). However, efforts to identify the allopeptide(s) presented by these HLA-B27 allomorphs has remained elusive. Here we utilise a combined immunoproteomics and combinatorial peptide library (CPL) approach for allopeptide discovery. For immunoproteomics, HLA-B27/peptide complexes from transfected C1R cells were captured by immunoaffinity chromatography, fractionated by reversed-phase high-performance liquid chromatography (RP-HPLC) and screened for immunogenicity against a reporter cell line (SKW3) expressing the OTN5 TCR. The composition of the active peptide containing fraction was determined by mass spectrometry and further validated using CPL screening assays to characterise HLA-B27-restricted peptides recognised by the OTN5 T cell. This study demonstrates utility of these two powerful technologies to enhance identification of naturally presented allopeptides.

P.C3.04.16

Stem cell and novel neurotrophic factor therapies to promote functional outcome in limb transplantation

S. K. Salgar, *J. Weiss;*
Madigan Army Medical Center, Tacoma, United States.

Introduction: Currently, sensory/motor function recovery following limb transplantation is suboptimal. We investigated whether adult Mesenchymal Stem Cells(MSCs), Granulocyte-Colony Stimulating Factor(G-CSF), and Dihexa can improve limb functional recovery in a rat sciatic nerve transection/repair model. **Materials and Methods:** Under general anesthesia (Lewis rat) the sciatic nerve was identified, transected, and repaired/approximated using 10-O sutures. MSCs(2 million), G-CSF(50-100μg/kg), (Dihexa 2-4mg/kg) and/or Vehicle were administered locally, i.v./i.p., and to gastrocnemius muscle. Sensory/Motor functions of the limb were assessed. **Results:** MSCs expanded *ex vivo* were CD29+, CD90+, CD34-, CD31-, MHC Class I+, Class II- and multipotent. At two weeks post-nerve repair, total sensory function (peroneal, tibial, saphenous, sural) in all groups was ~1.8 on a scale of Grade 0-3(0=No function; 3= Normal function); however, peroneal nerve function was ~ 2.8(n=6/group). At eight weeks, sensory function was restored to nearly normal ~2.8 in MSC+G-CSF and MSC+Dihexa groups but not in MSC+vehicle control(~2.3). At eight weeks, motor function as determined by walking foot print grades 0-4 (0=no print; 4=heel plus 4-5 toe prints) was 3.2±1, 2.5±1.4, and 1.8±1.2; and at 16 weeks 3.0±0.9, 3.0±0.8, and 2.0±0.6 in MSC+G-CSF, MSC+Dihexa and MSC+vehicle groups, respectively. Motor function improved($P<0.05$) in G-CSF and Dihexa groups. G-CSF or Dihexa administration to gastrocnemius mitigated muscle atrophy and flexion contractures, significantly. In a parallel study with tibial nerve transection, MSC improved($P<0.05$) nerve regeneration and myelination(n≥6). **Conclusions:** MSC with G-CSF or Dihexa therapies appear to be promising to promote limb functional recovery following sciatic nerve injury, and warrants further testing in limb transplant model.

P.C3.04.18

The number of circulating immature/transitional B cells correlates with the type 3 innate lymphoid cells in hematopoietic stem cell-transplanted patients with acute graft-versus-host disease

Z. I. Komlósi[1,2,3], *Z. Pós*[1], *N. Lupsa*[1], *G. Barna*[4], *P. P. Reményi*[5], *G. Tatai*[5], *G. Kriván*[5], *N. Kovács*[6], *T. Masszi*[5], *G. Losonczy*[7], *C. A. Akdis*[8,3];
[1]*Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary,* [2]*Swiss Institute of Allergy and Asthma Research (SIAF),University of Zurich, Davos, Switzerland,* [3]*CK-CARE: Christine Kühne Center for Allergy Research and Education, Davos, Switzerland,* [4]*1st Dept. of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary,* [5]*Department of Hematology and Stem Cell Transplantation, South Pest Centrum Hospital, Budapest, Hungary,* [6]*Lung Health Hospital, Törökbálint, Hungary,* [7]*Department of Pulmonology, Semmelweis University, Budapest, Hungary,* [8]*Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, Switzerland.*

Activated type 3 innate lymphoid cells (ILC3s) are expanded after hematopoietic stem cell-transplantation (HSCT), and have a protective effect against graft-versus-host disease (GVHD). However, the role of various B cell populations in the pathomechanism of GVHD is controversial. We have recently shown that activated ILC3s can help B cell survival, proliferation and the differentiation of immature/transitional B (itB) cells with immune regulatory properties. The impact of the activated ILC3 populations on B cells after HSCT, and their role in the prevention of GVHD have not been investigated thus far. Therefore, we aimed to examine the interrelation of ILC3s and itB cells in acute GVHD. Twenty patients of less than 60 days post-HSCT were included in the study, out of which ten were suffered from acute cutaneous GVHD. Their peripheral blood samples were analyzed before initiation of the treatment. Peripheral blood $Lineage^-CD127^+CD161^+c\text{-}Kit^+$ ILC3s, and their NKp44 and CD69 expression, as well as $CD19^+IgD^+CD24^{high}CD38^{high}$ itB cells and their PD-L1 expression were measured by flow cytometry.

The prevalence of ILC3s was lower in acute GVHD patients compared to HSCT patients without GVHD. Both the absolute numbers and the percentages of ILC3s and itB cells correlated with each other, and the correlation was particularly strong in acute GVHD patients. Our results suggest that activated ILC3s may have a favorable influence on the early post-HSCT period via its itB cell-inducing capacity; and itB cells with regulatory properties may have a protective role against acute GVHD. Funded by Hungarian Paediatric Oncology Network.

P.C3.04.19

Clonal tracking of CD45RA-depleted donor T-lymphocytes after infusion in TCR alpha/beta depleted transplantation from unrelated and haploidentical donor

*V. E. Fomchenkova[1], E. A. Komech[2], S. Blagov[3], V. Zhogov[3], Y. B. Lebedev[2], D. M. Chudakov[2], M. A. Maschan[3], **I. V. Zvyagin**[2,3];*
[1]Lomonosov Moscow State University, Moscow, Russian Federation, [2]Shemiakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russia, Moscow, Russian Federation, [3]Dmitry Rogachev National Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation.

Low number of T-lymphocytes and extremely low TCR diversity early after alpha-beta T-cell depleted allogenic transplantation holds the risk of death from different pathogens, specifically from persisting and reactivating viruses. Transfer of antigen-experienced T-lymphocytes from donor to recipient early after HSCT can provide the protection from pathogens during the period of immunodeficiency. Fraction of CD45RA-depleted T-cells could potentially serve as the source of the antigen-experienced T-lymphocytes and successful implementation of such selective T-cell depletion was recently shown in the setting of both matched and mismatched HSCT.
Here we report the study of clonal dynamics of CD45RA-depleted donor T-lymphocyte fraction infused in low-dose in children after allogenic HSCT with alpha-beta T-cell depletion. High-throughput sequencing with molecular barcode-based data normalisation was used for TCR beta repertoire profiling of samples of patients' peripheral blood and CD45RA-depleted donor T-cells (DLI) to track the clonal dynamics of donor cells in patients during 1 year after HSCT.
Small number of both CD8+ and CD4+ T cell clonotypes from DLI were observed in peripheral blood of patients early after infusion and persisted during 6 months. Their proportion decreased along with reconstitution of T-cell repertoire diversity and T-cell count. Most expanded clonotypes in recipients' repertoire in the early timepoint, including virus-specific T-cell clonotypes, mostly originated from clonotypes which was low frequent or not detected in DLI.
This study was supported by RFBR grant #16-04-01881-a.

P.C4.01 Manipulation of tolerance - Part 1

P.C4.01.01

High sodium concentration modulates dendritic cells immune function

***S. AL-HAJJ**[1], A. GOUMARD[1,2], A. HERAUD[1], S. GEORGEAULT[3], J. BURLAUD-GAILLARD[3], C. HOARAU[1,2], P. GATAULT[1,2], M. BUCHLER[1,2], R. LEMOINE[1], C. BARON[1,2], J. HALIMI[1,2];*
[1]EA4245 Dendritic Cells, Immunomodulation & Transplantation, TOURS, France, [2]Nephrology, clinical immunology service, University Hospital Center Bretonneau, Tours, France, [3]Laboratory of cell biology & electronic microscopy, François Rabelais University, Medicine school, TOURS, France.

Introduction: Excessive Na intake is associated with hypertension, increased skin sodium concentration [Na+] (up to 200 mM) and dendritic cells (DCs) recruitment. However, high [Na+] effect on DCs immune function remains unclear. **Methods:** DCs derived from human blood monocytes were stimulated by LPS for 48h at different [Na+]. We studied DCs cytosqueleton modifications by electron and confocal microscopy, analyzed cell viability, expression of CCR7 chemokine receptor ,CD25, CD83, CD86 and CD80 markers, FITC-DEXTRAN endocytosis, reactive oxygen species (ROS) production by flow cytometry. We studied DCs CCL19-driven chemotaxis using transwell, secreted cytokines by ELISA, MAP Kinase and SGK1 expression by western blot. **Results:** At high [Na+] of 200 mM as compared to normal [Na+] 140 mM, DCs viability was maintained (over 84.5±5%). DCs cytosqueleton displayed a more elongated aspect. CCR7, CD25, CD83 CD80 and CD86 expression significantly decreased (-59.3±15.3%, -67.7±32.3%, -60.4±14.5%, -25±20.8%, -13.6±10.3%, respectively, $p<0.0001$). There were less ROS production (-36.1±12.3%, $p<0.005$) and a persistence of endocytosis capacity (+108.3±44.6%, $p<0.005$) with a decreased CCL19-driven chemotaxis (-49.7±25.6%, $p<0.0005$). Cytokines measurement showed a reduced secretion of IL-12p70, IL-6 and IL-23 (-78.5±9%, -66.6±9.2%, -90.4±7.12%, respectively, $p<0.0001$) and an increase of IL-10 and TGF-β (+111±51%, +191.1±110.4%, respectively, $p<0.005$). Higher [Na+] lead to a reduced phosphorylation of p38 (-43.13±25%, $p<0.03$), higher ERK 1⁄2 phosphorylation (+30.57±11.6%, $p<0.007$) and greater SGK1 expression (+35.07±11.5%, $p<0.01$). **Conclusions:** High [Na+] concentration downregulates inflammatory LPS treated DCs immune response, through SGK1 and MAP Kinase related mechanisms. However, the implication of these changes in the development of hypertension remains unknown.

P.C4.01.02

A pro-inflammatory role for CD70 on human regulatory T cells

***R. Arroyo Hornero**, K. Wood, F. Issa, J. Hester;*
University of Oxford, Oxford, United Kingdom.

Introduction: The CD27-CD70 costimulatory receptor-ligand pair belongs to the TNFR superfamily. Ligation of CD27 on T cells with CD70 on APCs promotes T cell survival, controls $CD4^+$ T cell subset differentiation and it is essential for the generation of antigen-specific T cell immunity. CD70 is also expressed by T cells upon activation, although the role of T cell-expressed CD70 is unknown. We have previously shown that CD27 expression on human Tregs correlates closely with suppressive potency. In this study, we hypothesised that the CD27/CD70 axis could be exploited for regulating the balance between pro-inflammatory and regulatory T cell responses.
Materials and Methods: $CD4^+$ Tconv and $CD4^+CD127^{low/-}CD25^+$ Tregs were flow sorted from healthy donors PBMCs and activated for 14 days *in vitro* via αCD3/αCD28 stimulation in the presence of IL-2. Cells were then flow sorted according to CD27 and CD70 and expanded for 14 days prior to analysis.
Results: Human Tregs differentially altered CD27 and CD70 surface expression upon *in vitro* activation, resulting in two distinct subpopulations ($CD27^+CD70^-$ and $CD27^-CD70^+$). Functional *in vivo* and *in vitro* assays revealed that suppressive activity was confined to $CD27^+CD70^-$ Tregs, while $CD27^-CD70^+$ Tregs promoted T cell proliferation and produced higher levels of IL-17A. Stimulation or blockade of CD27 signalling did not affect Treg suppressive activity. In contrast, blocking CD70 on Tregs significantly enhanced their suppressive potency, abolishing the pro-inflammatory effect of $CD70^+$ Tregs.
Conclusions: This study reveals for the first time that $CD70^+$ Tregs provide stimulatory signals by ligating CD70 to CD27 on T cells.

P.C4.01.03

The effects of IFN-gamma on the expansion of suppressive B cells and their action

***P. Bohacova**[1,2], E. Javorkova[1,2], B. Hermankova[1,2], J. Kossl[1,2], A. Zajicova[1], V. Holan[1,2];*
[1]Institute of Experimental medicine, Prague, Czech Republic, [2]Faculty of Science, Prague, Czech Republic.

Introduction: Besides production of antibodies, B cells can also regulate immune response in the antibody-independent manner. Mechanisms of action of suppressive B cells are mainly the production of IL-10 or the expression of surface inhibitory molecules, such as FasL or PD-L1. In this study we have been aiming to characterize modulatory effects of IFN-γ on the development of suppressive action of B cells.
Materials and Methods: $CD19^+$ B cells were prepared using MACS and stimulated with LPS or LPS and IFN-γ for 48 hours. These cells were harvested and cocultivated with peritoneal macrophages for additional 48 hours. Then macrophage expression of costimulatory molecules was determined by flow cytometry and the cytokine production was evaluated by ELISA. Pretreated macrophages were used for the stimulation of CFSE labeled T cells to measure T cell proliferation by flow cytometry.
Results: B cells stimulated with LPS and IFN-γ produced increased concentrations of IL-10 and also expressed higher levels of the genes for FasL and PD-L1. Macrophages, which were cocultivated with B cells stimulated with LPS and IFN-γ, showed decreased expression of costimulatory molecule CD86 and reduced production of IL-6. These macrophages displayed also decreased ability to stimulate proliferation of activated $CD8^+$ T cells.
Conclusions: The results have shown that IFN-γ enhances activation of suppressive functions of B cells which have the ability to inhibit immune response through their effect on macrophages. The possibility to modulate regulatory B cells may have an impact for their use in a clinical setting.

P.C4.01.04

Early Onset Type 1 diabetes and typical juvenile diabetes are distinct clinical and genetic entities

***I. Caramalho**[1], P. Matoso[1], D. Sobral[1], J. Costa[1], D. Ligeiro[2], A. Fitas[3], C. Limbert[3], C. Penha-Goncalves[1], J. Demengeot[1];*
[1]Instituto Gulbenkian de Ciência, Oeiras, Portugal, [2]Centro de Sangue e Transplantação de Lisboa, Instituto Português de Sangue e Transplantação, Lisboa, Portugal, [3]Hospital D. Estefânia, Centro Hospitalar de Lisboa Central, Lisboa, Portugal.

Insulin-dependent Type 1 diabetes (T1D) results from the immune-mediated destruction of insulin-producing pancreatic beta cells, triggered by the interplay between environmental and genetic factors. Susceptibility alleles include Human Leukocyte Antigen (HLA) class II haplotypes and over 100 genetic variants located in more than 50 genetic loci.
In the last decades, an alarming increase in the incidence of T1D affecting preschool children has occurred in Western countries, an unexplained epidemiological concern. We set out to determine whether Early Onset (EO)T1D is clinically and genetically distinct from the typical juvenile T1D.
We established a cohort of 100 EOT1D patients (age of onset ≤5 years) and performed their detailed clinical characterization, including familial history of autoimmune diseases. DNA samples were processed for HLA typing, Single Nucleotide Polymorphisms (SNP) genotyping and Whole Exome Sequencing.

Our clinical analysis evidence that EOT1D patients present with a more severe disease when compared to children with later onset of disease (age of onset >9 years). Moreover, a subset of EOT1D patients presents poly- or multiple- autoimmunity and a familial history of autoimmune aggregation. Our genomic analysis reveals EOT1D differs from typical juvenile T1D, at the HLA and at several of the 118 SNP analyzed. Moreover we identified novel genetic variants likely causal in the aetiology of EOT1D.
Our findings support the notion that EOT1D and juvenile T1D are distinct clinical entities with specific genetic architecture, and should feed the development of predictive diagnostic and the implementation of early preventive measures.

P.C4.01.05

Tolerogenic effects of ethyl pyruvate on dendritic cells

N. Djedovic[1], *M. Mansilla*[2,3], *B. Jevtic*[1], *J. Navarro-Barriuso*[2,3], *S. Stanisavljevic*[1], *E. Martínez-Cáceres*[2,3], *D. Miljkovic*[1];
[1]Institute for Biological Research "Sinisa Stankovic", Belgrade, Serbia, [2]Immunology Division, Germans Trias i Pujol University Hospital and Research Institute, Campus Can Ruti, Badalona, Spain, [3]Department of Cellular Biology, Physiology and Immunology, Universitat Autònoma de Barcelona, Cerdanyola del Vallés, Barcelona, Spain.

Introduction: Dendritic cells (DC) are professional antigen presenting cells that have a key role in shaping the immune response. Tolerogenic DC (tolDC) have immuno-regulatory properties and they are a promising prospective therapy for multiple sclerosis (MS). Ethyl pyruvate (EP) is a redox analogue of dimethyl fumarate (Tecfidera), a drug for MS treatment. We have recently shown that EP ameliorates experimental autoimmune encephalomyelitis (EAE), a MS animal model, and that it induces tolerogenicity in mice DC. Here, we expanded our study on human DC.
Methods: Monocyte-derived DC are obtained from MS patients and healthy individuals in the presence of GM-CSF and IL-4 for 6 days. EP is applied to the cultures on days 2 and 4, while maturation stimulus (TNF, IL-1β, PGE_2) is added on day 4 of cultivation.
Results: Phenotypic analysis has shown that DC treated with EP (tEPDC) have significantly reduced levels of molecules required for T cell activation such as CD86, CD83 and HLA-DR whereby CD11c expression and viability of DC were not affected. Further, tEPDC restrained proliferation and modulated cytokine production of allogeneic lymphocytes.
Conclusion: These results demonstrate that ethyl pyruvate has the ability to direct human DC towards tolDC. *In vivo* study on application of tEPDC in EAE and detailed molecular characterisation of these cells are warranted. These steps should complete pre-clinical studies on tEPDC as potential MS therapy. Funding: MPNTR Republic of Serbia, OI173013/ OI173035. Project PI14/01175, PI17/01521, integrated in the Plan Nacional de I+D+I and co-supported by the ISCIII-Subdirección General de Evaluación and the FEDER.

P.C4.01.06

The effect of CD6 targeting in T cell function and Immunopathology

R. F. Freitas[1,2], *S. Almeida*[3,4], *S. Perez*[5], *L. Padron*[5], *K. Leon*[5], *L. Graça*[1,2];
[1]Instituto de Medicina Molecular, Lisboa, Portugal, [2]Instituto Gulbenkian de Ciência, Oeiras, Portugal, [3]Post-Graduate Program in Infectious Diseases, Federal University of Espirito Santo, Vitória, Brazil, [4]Department of Social Medicine, Center of Health Sciences, Federal University of Espírito Santo, Vitória, Brazil, [5]Centro de Inmunologia Molecular, Habana, Cuba.

Introduction: CD6 transmembrane glycoprotein is a cell surface receptor (with three extracellular domains and a long cytoplasmic tail) from the scavenger receptor cysteine rich protein superfamily, expressed in thymocytes, mature T cells, B1a cells and subsets of natural killer cells. One of its three ligands is the activated leukocyte adhesion molecule (ALCAM) that binds to domain 3, thereby affecting T cell activation, proliferation, immune synapse formation and transmigration across the blood brain barrier (BBB). Genome wide association studies identified CD6 as a susceptibility locus for Multiple Sclerosis (MS) and, in clinical trials for psoriasis a humanized anti-CD6 Mab (T1h), has already been tested with promising results. Investigation of CD6-targeting in MS has been hampered by lack of adequate murine reagents. We tested a "murinized" variant of T1h in a mouse model of MS, in order to establish the therapeutic value of CD6-targeting for prevention of autoimmune neuroinflammation. Materials and Methods: Experimental autoimmune encephalomyelitis (EAE) was induced in C57BL/6 mice with subcutaneous injection of MOG_{35-55} emulsified in CFA. Intravenous injection of Pertussis was administered on day of immunization and two days later. The experimental groups received increasing doses of anti-CD6 Mab and they were followed for 30 days. Results/Conclusions: Our data show CD6 targeting led to a delay in the EAE onset, compared to control animals treated with an isotope control. Subsequent studies are required to establish the mechanism leading to the protective effect of CD6-targeting.

P.C4.01.07

Lipidoid-polymer hybrid nanoparticles loaded with anti-TNF siRNA suppress inflammation after intra-articular administration in a murine experimental arthritis model

M. A. A. Jansen[1], *L. H. Klausen*[2], *K. Thanki*[2], *H. Franzyk*[2], *H. Mørck Nielsen*[3], *W. van Eden*[1], *M. Dong*[2], *F. Broere*[1], *C. Foged*[3], *X. Zeng*[3];
[1]Utrecht University, Utrecht, Netherlands, [2]Aarhus University, Aarhus, Denmark, [3]Copenhagen University, Copenhagen, Denmark.

Introduction: Rheumatoid arthritis (RA) is an autoimmune disease which is characterized by chronic inflammation in the joint. RNA interference (RNAi) therapy is a promising way to target gene silencing locally and suppress excessive inflammation. However, efficient delivery of small interfering RNA (siRNA) into a cell is problematic. Therefore, there is a need for new and safe delivery systems. Recently lipid-polymer hybrid nanoparticles (LPNs) for nucleic acid delivery are developed, since these LPNs are less immunogenic compared to other particles.
Methods: We studied the siRNA delivery of the lipidoid-modified poly (DL-lactic-co-glycolic acid) LPNs and stable nucleic acid lipidoid particles (SNALPs) in the murine macrophage cell line RAW 264.7, and investigated their structure-function relationship. Furthermore, we tested the therapeutic capacities of these nanoparticles containing anti-tumor necrosis factor (TNF) siRNA in a murine arthritis model.
Results: Results indicate pathway-specific differences in delivery of siRNA to macrophages between LPNs and SNALPs. Both particles were taken up by micropinocytosis but the SNALP take-up by macrophages also showed signs of clathrin-mediated endocytosis. Next to this, we show that LPNs and SNALPs loaded with anti-TNF siRNA (1 µg) mediate sequence-specific suppression of inflammation in a murine experimental arthritis model upon intra-articular administration.
Conclusion: The results from this study show that functional anti-TNF siRNA encapsulated by LPNs or SNALPs can be delivered both *in vitro* and *in vivo*. The therapeutic effectivity from LPNs and SNALPs containing anti-TNF siRNA indicates that this is a promising therapy for rheumatoid arthritis and possibly other chronic inflammatory diseases.

P.C4.01.08

Tolerogenic potential of dendritic cells obtained from autoimmunity prone and resistant rats

B. Jevtic[1], *N. Djedovic*[1], *S. Stanisavljevic*[1], *G. Timotijevic*[2], *M. Momcilovic*[1], *M. Mostarica Stojkovic*[3], *D. Miljkovic*[1];
[1]Institute for Biological Research, Belgrade, Serbia, [2]Institute of Molecular Genetic and Genetic Engeenering, Belgrade, Serbia, [3]Institute of Microbiology and Immunology, Belgrade, Serbia.

Experimental autoimmune encephalomyelitis (EAE) is a widely used animal model of multiple sclerosis, a chronic, inflammatory and neurodegenerative disease of the central nervous system (CNS). Dendritic cells are essential for the initiation, propagation and for regulation of the autoimmune response against the CNS. One of the main approaches in multiple sclerosis immunotherapy is the application of tolerogenic dendritic cells (tolDC). The aim of this study was to compare the tolerogenic potential of DC obtained from autoimmunity-prone (Dark Agouti, DA) and resistant (Albino Oxford, AO) rats. For this purpose, DC were generated from rat bone marrow of non-immunized and EAE-immunized rats and propagated towards tolDC in the presence of GM-CSF and vitamin D3. tolDC were characterized phenotypically by cytofluorimetry. mRNA expression of various genes was determined by RT-qPCR and cytokines generation by ELISA. Results have shown that AO and DA rat tolDC differ in expression of various mRNA and production of cytokines, emphasizing a stronger tolerogenic potential of tolDC from AO compared to DA rats. Furthermore, tolDC from these strains differed in response to EAE-immunization. The observed differences between tolDC of rats prone and resistant to the CNS autoimmunity call for a similar study in humans. Acknowledgements: This study was supported by grants from the Ministry of Education, Science and Technological Development, Republic of Serbia (173035, 173013, 175038).

P.C4.01.09

Interleukin-10 transfected dendritic cells-induced Treg cells prompt an allogenic tolerance *in vitro* in mice C57BL/6 but not in mice CBA

J. Khantakova, *V. Tereschenko, V. Kurilin, J. Lopatnikova, A. Silkov, N. Knauer, A. Maksyutov, S. Sennikov;*
Federal State Budgetary Institution "Research Institute of Fundamental and Clinical Immunology", Novosibirsk, Russian Federation.

Introduce: Regulatory T-cells (Treg cells) are maintaining the tolerance to self- and foreign antigens. We explored the feasibility of inducing Treg cells by using IL-10 transfected dendritic cells (DCpIL-10). Materials and methods: C57Bl/6 and CBA female mice were obtained from the breeding facility of the Institute of Cytology and Genetics (Novosibirsk, Russia). DCs were generated from the bone marrow monocyte pool with added rmGM-CSF and rmIL-4. The cells were electroporated on days 3 with plasmid pmax-pIL-10 encoding murine IL-10 (pIL-10). Treg cells in splenocyte culture were induced by using DCpIL-10.The phenotypic indicators of the DCs (CD11c, H2, costimulatory molecules) and Treg (CD4, CD25, FoxP3, IL-10) cells were assessed on a FACSVerse flow cytometer. DCpIL-10 effects on allogenic tolerance were investigated by MLR. Results: C57Bl/6 and CBA mice were different in initial percentage of Treg cells and IL-10 production. In C57Bl/6 mice DCpIL-10 guided naïve CD4+ T cells differentiation to Treg cells. This is accompanied by increase IL-10 production and decrease proliferation on allo-splenocytes. In CBA mice DCpIL-10 failed to Treg cells induction, splenocytes proliferation inhibition and IL-10 production.
Conclusion: Initial count of Treg cells and IL-10 production influence to the Treg cells induction. DCpIL-10-induced Treg cells prompt an allogenic tolerance *in vitro* only in C57Bl/6 mice but not CBA mice. The research carried out with the support of the RSF. Agreement №16-15-00086 (11.01.2016).

P.C4.01.10

Interaction of antigen-presenting cells and T cells is modulated by exosomes carrying miRNA-150

K. Nazimek[1,2,3]*, E. Bustos-Morán*[2]*, N. Blas-Rus*[2]*, P. W. Askenase*[3]*, F. Sánchez-Madrid*[2]*, K. Bryniarski*[1]*;*
[1]Department of Immunology, Jagiellonian University Medical College, Krakow, Poland, [2]Department of Immunology, Hospital de la Princesa, Autonomous University of Madrid, Madrid, Spain, [3]Section of Allergy and Clinical Immunology, Yale University School of Medicine, New Haven, United States.

Introduction. Mouse contact hypersensitivity reaction (CHS) is suppressed by T CD8+ cell-derived miRNA-150 carried by exosomes coated with antigen-specific antibody light chains [J Allergy Clin Immunol 2013;132:170-81]. These exosomes target antigen-presenting macrophages that in turn suppress CHS effector cells [Immunology 2015;146:23-32]. Nanovesicle-mediated interactions between cells at the site of antigen presentation were recently examined, showing the passage of CD63-positive exosomes from intact T to B lymphocytes forming the conjugates (immune synapses) [Nat Commun 2011;2:282]. Our current study aimed at investigating the impact of exosomes carrying miRNA-150 on the vesicle-dependent intercellular interactions at the immune synapse.
Methodology. Jurkat T cells and Raji B cells were transfected with CD63-GFP or CD81-GFP plasmids by electroporation and then incubated with miRNA-150-containing exosomes. Superantigen-stimulated formation of conjugates by Jurkat T cells and Raji B cells was then assessed in fluorescence confocal microscopy and intercellular transmission of vesicles as well as T cell activation was analyzed cytometrically.
Results. The polarization of Raji B-cell, CD81-GFP-positive multivesicular bodies towards the site of CD3 accumulation in T cells (immune synapse) was observed. Further, miRNA-150-carrying exosomes induced transfer of CD81-positive, but not CD63-positive, vesicles from Raji B cells to Jurkat T cells. Afterwards, Jurkat T cells were characterized by lower CD69 activation marker expression.
Conclusions. Exosomes carrying miRNA-150 induce the transmission of vesicles from antigen-presenting cells to T cells, impairing their activation at the immune synapse. Supposedly, this mechanism reflects the action of antigen-presenting macrophages on effector T lymphocytes during the suppression of CHS activated by miRNA-150-carrying exosomes.

P.C4.01.11

Role of NK cells in the onset of Rheumatoid Arthritis.

S. Pascual-García*, P. Martínez-Peinado, A. López-Jaén, F. Navarro-Blasco, G. Peiró, J. Sempere-Ortells;*
University of Alicante, Alicante, Spain.

Introduction. Rheumatoid arthritis (RA) is a Th1 systemic autoimmune disease, whose incidence in the Northern Hemisphere countries is from 0.5% to 1%. It is characterised by an inflammation of the synovium leading the destruction of both the cartilage and the bone, diminishing the patients' quality of life. The aim of this study is to determine the prognostic value of the expression of IgG Fc receptors and markers of naïve/effector cells in the different leukocytes subpopulations. Materials and methods. Anticoagulated blood samples from 20 healthy donors and 7 RA *de novo* diagnosed patients were marked with anti-CD16, anti-CD32, anti-CD64, anti-CD45RO and anti-CD45RA monoclonal antibodies (BD and eBioscience). Afterwards, data were analysed using FACSCanto I (BD). Results. A significant increase in the expression of CD64+, CD32+CD64+, and CD16+CD32+CD64+ NK cells from RA patients was observed when compared with healthy donors (p=0.029, p=0.0371 and p=0.0187, respectively). However, percentages of CD16+ granulocytes and CD16+CD64 monocytes from these patients were lower (p=0.0007 and p=0.0046, respectively). On the other hand, we also found a remarkable increase in the percentage of CD45RO+ NK cells from RA patients (p=0.05), as compared with healthy volunteers. Conclusion. NK cells from *de no*vo diagnosed RA patients show a mature phenotype and an increased expression of the IgG Fc receptors. The author has a grant supported by Ministerio de Educación, Cultura y Deporte with reference FPU14/01984.

P.C4.01.12

Generation of human Breg-like phenotype with regulatory function *in vitro* with bacteria-derived oligodeoxynucleotides

J. Gallego-Valle, V. Perez-Fernandez, R. Correa-Rocha, ***M. Pion****;*
Instituto de Investigacion Sanitaria Gregorio Marañon, Madrid, Spain.

Introduction: Regulatory B cells (Breg) participate in the auto-tolerance maintenance and immune homeostasis. Despite their impact on many diseases and due to the difficulty to define them, knowledge about their origin and their physiological inducers are still unclear. The incomplete knowledge about the generation of Breg and their limited numbers in periphery difficult the development of Breg-based therapy. Therefore, identifying factors that promote their development would allow ex-vivo production of large amounts of Bregs in order to create new immunotherapy. **Materials and Methods:** We tested the capacity of several cytokines (IL-1β, GM-CSF and CD40L) and bacteria-derived oligodeoxynucleotides (CpG-ODN), alone or in combination, to generate B cells with regulatory phenotype and function in an *in vitro* model. By flow cytometry we followed Breg-like phenotype in human primary stimulated B cells. Moreover, by co-culture experiments we followed suppressive ability of such cells to suppress PBMC proliferation. **Results:** We have demonstrated that the Breg-associated phenotypes were heterogeneous between one to another stimulation conditions. However, the expression of others markers related to Breg was increased such as IL-10, CD80, CD86, CD71, PD-1 and PD-L1 when cells were stimulated with CpG alone or in combination. Moreover, stimulated B cells presented a suppressive function on autologous activated PBMC proliferation. **Conclusions:** This work demonstrated the feasibility to induce functional Breg-like cells *in vitro* and then open the way to produce Breg-like as a potential future cellular therapy.

P.C4.01.13

Arthritogenic peptides presented by tolerogenic dendritic cells are able to reprogram effector CD4+ T cell responses from rheumatoid arthritis patients

E. C. Rivas Yañez[1]*, J. Maggi*[1]*, O. Aravena*[1]*, K. Schinnerling*[1]*, L. Soto*[2]*, O. Neira*[3]*, D. Catalán*[1]*, J. C. Aguillón*[1]*;*
[1]Laboratorio de Enfermedades Autoinmunes e Inflamatorias, Programa Disciplinario de Inmunología, Santiago, Chile, [2]Unidad de Tratamiento del Dolor, Hospital Clínico, Universidad de Chile, Santiago, Chile, [3]Hospital Del Salvador, Santiago, Chile.

Introduction: Rheumatoid arthritis (RA) is a disabling autoimmune disease generating joint inflammation. A therapeutic approach is the administration of tolerogenic dendritic cells (tolDCs), able to modulate autoreactive T cell responses and re-establish self-tolerance. The selection of appropriate autoantigens to be presented by tolDC is a critical element in the design of tolDC-based therapies. We aimed to identify immunodominant autoantigen peptides and elucidate the mechanisms by which tolDCs challenged with these self-peptides modulate lymphocyte responses. **Methods:** Peripheral blood mononuclear cells (PBMCs) were challenged with 12 autoantigen peptide candidates and reactivity of CD4+ T cells was evaluated based on expression of CD25, CD69, IFN-γ and TNF-α. Peptide-pulsed tolDCs generated from RA patient-derived monocytes were cocultured with autologous CD4+ T cells or peptide-specific T cell lines. Modulation of T cell phenotype and cytokine profile was assessed by flow cytometry. **Results:** From 12 peptide candidates, recently isolated by our group through natural processing of synovial proteins, we identified 6 immunodominant peptides, derived from calreticulin, vimentin, citrullinated vimentin and fibrinogen, which generated inflammatory CD4+ T cell responses in 27-39% of RA patients. While mature DCs, pulsed with these autoantigen peptides, induced CD4+ T cell proliferation and pro-inflammatory cytokine production in cocultures, autoantigen peptide-loaded tolDCs were able to modulate this response, promoting hyporesponsiveness of autoreactive T cells. **Conclusion:** We identified 6 novel immunodominant self-peptides which are recognized by autoreactive T cells from RA patients and are therefore appropriate autoantigens for loading of tolDCs used as cell-based therapy of RA. Support: Fondef ID15I10080 and Fondecyt 1181853.

P.C4.01.14

Inhibitory oligodeoxynucleotides induce an alternative activation state in human plasmacytoid dendritic cells

J. Ruben*, S. van der Kooij, C. van Kooten;*
Div. of Nephrology and Transplant Medicine, Dept. of Medicine, Leiden University Medical Center, Leiden, Netherlands.

Plasmacytoid dendritic cells (pDC) recognize CpG oligodeoxynucleotides (ODN) via TLR9, leading to the production of type I interferons (IFNα) and enhanced antigen presenting cell functions. Mammalian telomeres and commensal bacteria contain sequences which have been described as inhibitory ODN (iODN), since they act as potent antagonists of TLR9 activation. As such, iODN treatment of animal (autoimmune) models was shown to induce immune regulation. Here, we confirm that iODN can dose-dependently inhibit IFNα production by human pDC following TLR9 ligation by either CpG or Cytomegalovirus. In contrast to the IFNα production, TLR9-induced phagocytosis could not be inhibited by iODN. In fact, iODN treatment induced phagocytosis by pDC in the absence of TLR9 agonists, suggestive for the induction of active signaling. Although pDC treatment with iODN did not lead to the production of detectable levels of IFNα, low levels of TNFα and IL-6 could be detected. Interestingly, where CpG-activated pDC increased their capacity to cross-present antigen, iODN-activated pDC strongly enhanced indirect ($CD4^+$) antigen presentation. Moreover, activation of pDC with iODN strongly upregulated the costimulatory molecules CD80 and CD86. Concordantly, iODN-activated pDC were capable of inducing a robust allogeneic T cell proliferation. Despite the strong increase in T cell proliferation, IFNγ levels were >4-fold lower compared to CpG-activated pDC, whereas IL-5 was produced at higher levels. In conclusion, we show that iODN directly impact pDC function and should be classified as a group of novel pDC activating ligands, capable of inducing an alternative activation state as compared to CpG ODN.

P.C4.01.15

Dendritic cells transfected by H-2K epitopes induce functional Tregs and tolerance *in vitro*.

V. Tereshchenko, J. Khantakova, V. Kurilin, A. Silkov, J. Schevchenko, J. Lopatnikova, A. Maksyutov, ***S. Sennikov****;*
Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation.

Introduce: CD4+CD25+Foxp3+Treg cells are necessary component for establishment and maintenance of immune tolerance. We explored the capacity of tolerogenic dendritic cells (tolDCs) transfected by DNA plasmid encoding H-2K epitopes of CBA mice to generate functional Treg cells for tolerance induction.
Materials and methods: DCs were generated from C57Bl/6 mice bone marrow monocyte pool with addition of rmGM-CSF and rmIL-4 without maturation factors. DCs were electroporated by 2 different plasmids: pGVHD encoding H-2K locus epitopes of CBA mice (DCs-pGVHD) and control plasmid (DCs-p5). Electroporated DCs were cultured with autologous splenocytes to generate functional Tregs. The phenotype properties of the DCs (CD11c, H2, CD80, CD86, CD83, and CD40) and Tregs (CD4, CD25, FoxP3, and IL-10) were investigated using flow cytometry. Functional capacity of transfected DCs and Tregs to induce tolerance was investigated by mixed lymphocyte reaction.
Results: DCs-pGVHD represent a tolerogenic phenotype (increase H2-B and decrease CD86, CD80) compared to DCs-p5. DCs-pGVHD induce FoxP3 and IL-10 expression by CD4+CD25hi T-cells in autologous splenocytes cultures. DCs-pGVHD-treated autologous splenocytes cultures showed significantly lower proliferation rate in response to CBA mice splenocytes when compared with control cultures.
Conclusion: DCs electroporated by DNA plasmid encoding H-2K epitopes of CBA mice induce functional Treg cells in splenocytes cultures and guide tolerance *in vitro*.
The research carried out with the support of the RSF. Agreement №16-15-00086 (11.01.2016).

P.C4.01.16

Hemoglobin is preferentially inflammatory, antigenic and immunogenic in lupus

H. Sharma*[1], S. Jain[1], A. Bose[1], B. Bastia[2], R. Sachdeva[1], A. K. Jain[2], R. Pal[1];*
[1]National Institute of Immunology, Delhi, India, [2]National Institute of Pathology, Delhi, India.

Hemolysis-associated anemia is characteristic of diseases such as atherosclerosis, lupus, malaria, and leishmaniasis; the toxic effects of free hemoglobin (Hb) have been extensively described. This study was based on the premise that release of this sequestered, inflammatory molecule can result in deleterious immunological consequences, particularly in the context of pre-existing lupus. PBMCs derived from SLE patients preferentially secreted a variety of inflammatory cytokines in response to Hb, and IgG anti-Hb responses were detected in the sera of lupus patients. Lupus-prone mice exhibited heightened plasma Hb levels, and Hb triggered the preferential release of lupus-associated cytokines from splenocytes derived from aging mice. Additionally, Hb induced the release of IL-17A, IL-12 and IL-8 from plasmacytoid dendritic cells, while also eliciting the release of a spectrum of inflammatory cytokines from purified CD8 T cells, CD4 T cells and B cells derived from such mice. Anti-Hb B cell precursor frequencies were heightened in lupus-prone mice, which exhibited increased titers of anti-Hb antibodies in serum and in kidney eluates. Hb interacted with lupus-associated autoantigens extruded during apoptosis, and co-incubation of Hb and apoptotic blebs had maturation-inducing effects on bone marrow-derived dendritic cells from lupus-prone mice. Immunization of such mice with Hb induced antigen spreading to lupus-associated moieties; increased complement deposition in the kidneys and enhanced-onset glomerulosclerosis were observed. Hb therefore elicits increased inflammatory responses from a variety of cell types, demonstrates both antigenicity and immunogenicity, and triggers specific immuno-pathological effects in a lupus milieu.

P.C4.01.17

Treatment of collagen-induced arthritis mice model with genetically modified tolerogenic dendritic cells

I. Yilmaz*[1], M. Karacay[1], G. Guvenc[2], E. Uz[3], F. Budak[4], F. Ersoy[3], M. Yalcin[5], H. B. Oral[4];*
[1]Department of Medical Immunology, Institute of Health Sciences, Uludag University, Bursa, Turkey, [2]Department of Veterinary Physiology, Institute of Health Sciences, Uludag University, Bursa, Turkey, [3]Department of Molecular Biology & Genetics, Faculty of Arts & Sciences, Uludag University, Bursa, Turkey, [4]Department of Immunology, Faculty of Medicine, Uludag University, Bursa, Turkey, [5]Department of Physiology, Faculty of Veterinary, Uludag University, Bursa, Turkey.

T cells activation has an important role in RA pathogenesis. Activation of T lymphocytes requires the co-stimulatory signals provided by antigen-presenting cells. In this study, to inhibit the activation of T lymphocytes in experimental arthritis, tolerogenic dendritic cells (tolDCs) were aimed to be obtained by the genetic modification of bone marrow-derived dendritic cells (BM-DCs). B7 co-stimulatory molecules expression were down-regulated with a gene construct encoding a modified cytotoxic T lymphocyte antigen 4 molecule (CTLA4-KDEL) which targets to the endoplasmic reticulum (ER). Mouse CTLA4 cDNA mammalian expression plasmid (pCMV/mCTLA4) was commercially provided from Sino Biologicals (China). The mCTLA4 gene was cloned into pCMV/myc/ER (Invitrogen, Life Technologies, USA). Plasmids subcloned into LeGo-iG2 (AddGene, USA) for lentiviral vector production. BM-DCs were non-viral and lentiviral transfected with CTLA4-KDEL and incubated for 48 hours. Flow cytometric analysis was performed using mouse monoclonal antibodies against CD80 and CD86 (Tonbo Biosciences, United Kingdom) and appropriate isotype controls. Furthermore, in vivo studies, tolDCs were transferred intraarticularly 3 weeks after collagen-induced arthritis (CIA) mice model and followed up for 4 weeks. It was observed that CD80/86 expression on the surface of BM-DCs significantly downregulated as tolDCs. Moreover, in vivo studies showed that tolDC treatment group significantly reversed the increase in the joint thickness and number of white blood cells compared with control groups. This study is supported by The Scientific and Technical Research Council of Turkey (TUBITAK-COST Project No: 114S354) under Cost Action BM1404. COST is supported by the EU Framework Programme Horizon 2020.

P.C4.02 Manipulation of tolerance - Part 2

P.C4.02.01

Cross-talk between the maternal endometrial micro-environment and tolerogenic dendritic cells

G. Amodio*[1], P. Panina-Bordignon[2], C. Semino[2], S. Gregori[1];*
[1]San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), IRCCS San Raffaele Scientific Institute, Milan, Italy, [2]Division of Genetics and Cell Biology, IRCCS San Raffaele Scientific Institute, Milan, Italy.

Successful pregnancy involves highly coordinated interactions between decidualized endometrial stromal cells and maternal immune cells to generate a receptive “maternal niche” for embryo implantation. DC-10 are a subset of dendritic cells, expressing HLA-G and secreting IL-10, which modulate T cell responses, promote tolerance, and accumulate in decidua during the first trimester of pregnancy. To assess whether DC-10 are critical components involved in favoring embryo implantation and successful pregnancy, we studied the in vitro crosstalk between endometrial stromal cells and DC-10. Immortalized human endometrial stromal cells (T-HESC) were *in vitro* decidualized with cAMP and medroxyprogesterone acetate for 7 days. During T-HESC decidualization, supernatants were collected at different time points and added during DC differentiation from peripheral blood $CD14^+$ monocytes. As control, DC-10 were differentiated from the same monocytes without T-HESC supernatants. Differentiated DCs were characterized by: i) expression of DC-10-associated markers; ii) cytokine profile; iii) ability to suppress a primary allogeneic response *in vitro*. Results showed that monocytes cultured with T-HESC-conditioned media collected at day 2 post-hormonal stimulation, differentiated into DC-10-like cells as demonstrated by the expression of CD14, CD141 and CD163, and display a cytokine secretion profile similar to that of DC-10. Moreover, DC-10-like cells suppress allogeneic $CD4^+$ T cell responses showing a functional parallel with DC-10 . These results support the hypothesis that the decidual micro-environment of early pregnancy sustains and promotes tolerogenic DC-10 differentiation. We are currently investigating the interactions of DC-10-like cells with the different immune players known to influence pregnancy outcome as NK cells.

P.C4.02.02

Milk protein-specific IgE and IgG4 in pediatric patients with food allergy

C. Kang*, Y. Yang, B. Chiang;*
Department of Pediatrics, Taipei, Taiwan.

Introduction: Food allergy is gaining attentions from the clinicians as it leads the development of atopic march. Milk proteins are the first foreign proteins an infant could encounter in life. We studied the prevalence and manifestation of milk-protein sensitized patients with the milk protein specific IgE and IgG4 levels.
Methods: We retrieved test results of serum allergen-specific tests (Mast and ImmunoCAPs, respectively). Any IgE level of specific food allergen higher then 2+ (range: 0~4+) in MAST IgE level higher than 0.70 kUA/L in ImmunoCAPs (class 2 out of class 0~6) was considered positive. In addition, milk protein-specific IgG4 will be also assessed.
Results: Total 1064 pediatric patients (55.6% of total 1914 patients) had Mast test, while 196 pediatric patients (6.61% of total 2966 patients) received ImmunoCAPs. The percentage positive for food specific IgE were 33.55% (n = 357) and 20.9% (n = 41) respectively for each tests. The age of Milk IgE(+) patients are significantly younger than the general food IgE(+) patients (2.83 ± 1.79 years vs 5.75 ± 3.92 years, p-value 0.005). Most of the milk IgE(+) patients presents with atopic dermatitis clinically, especially patients with higher titers.
Conclusions: The prevalence of milk protein sensitization was lower than what we expected, with the majority among children less than 5 years old. The age of milk IgE(+) patients is significantly younger than patients with other kinds of food IgE. The development of tolerance to milk protein over time might play a role in the reduction of milk protein-specific IgE.

P.C4.02.03

THEEFFECT OF RECOMBINANTPP 65 OF CMV ON FUNCTION AND MATURATION OF MICE DC

M. Karimi[1], *R. Yaghobi*[1], *Y. Nikmanesh*[1,2], *S. Shahmahmoodi*[1,2], *S. Marashi*[1,3];
[1]*Transplant Research Center, shiraz, Iran, Islamic Republic of,* [2]*Tehran University of Medical Sciences, Virology Department, Tehran, Iran, Islamic Republic of,* [3]*Tehran University Of Medical Sciences, Virology Department, Tehran, Iran, Islamic Republic of.*

The aim of the present study was to assess the impact of pp65 on the maturity and function of DCs. DCs were isolated and purified from the spleen of Balb/c mice. The HCMV-derived protein was exposed to isolated DCs. Then, DCs maturation was determined by using CCR7, CD86, CD40 and MHCII. The function of pp65-treated DCs was evaluated using the MLR assay. In addition, we measured transcriptional factors and T cell differentiation factors, including ROR-γ, GATA3, T-bet, and FoxP3, using Real time-PCR method. Cytokines levels INF-γ (Th1), IL4 (Th2), IL35 (Treg) IL17 (Th17), IL-22, TNF-α, and IL-6 were also determined by ELISA and flow cytometry. The phagocytosis rate in the group treated with pp65 was lower than the non-treated group, showing a significant difference. The levels of CD40, CD86 and CCR7 in the treatment group showed a significant increase compared with the non-treated group. The expression of ROR-γ and T-bet gene in the treatment group showed a significant increase compared with the non-treated group.The cytokine levels of IL-12, IL-6, IL-17, IL-22, and TNF-α showed a significant increase in the treated group as compared with the non-treated group, while a significant increase in the IFN-γ cytokine level was observed in pp65-treated groups as compared with the non-treated group. However, IL-4 and IL-35 cytokine levels were not detected in treated groups. Therefore, Such DCs can be used to induce immunity against CMV infection for CMV reactivation inhibition, particularly in immunosuppressed and transplanted individuals.

P.C4.02.04

Opioid analgesics differently modulate immune responses in mice

I. Filipczak-Bryniarska[1], *K. Nazimek*[2], ***M. Kozlowski***[1,2], *M. Wasik*[2], *K. Bryniarski*[2];
[1]*Department of Pain Treatment and Palliative Care, Jagiellonian University Medical College, Krakow, Poland,* [2]*Department of Immunology, Jagiellonian University Medical College, Krakow, Poland.*

Introduction. Immune cells commonly express opioid receptors and thus could be stimulated with opioids exerting immunomodulatory effects. Current research aimed at investigating the influence of various opioid analgesics on immune responses in mice under homeostatic conditions.
Methods. Mice were treated intraperitoneally with respective opioid drug (morphine, fentanyl, methadone, buprenorphine, oxycodone) for a week and, in some instances, skin-sensitized with hapten to induce contact hypersensitivity (CHS). Macrophage-induced humoral immunity was assessed in plaque-forming assay together with measurement of antibody titers in sera of mouse recipients of SRBC-pulsed macrophages from mice treated with different opioids. In addition, the effects of opioids on the production of reactive oxygen intermediates (ROIs), nitric oxide and cytokines by peritoneal macrophages along with expression of surface markers were estimated.
Results. Morphine administration significantly intensified CHS response in actively sensitized mice, while buprenorphine or oxycodone administration exerted the opposite effect. All tested opioids enhanced the release of proinflammatory cytokines, ROIs and nitric oxide by macrophages and altered their expression of antigen phagocytosis and presentation markers. Additionally, the inhibitory effect of morphine, fentanyl and methadone treatment on induction of humoral immunity by macrophages was demonstrated, while buprenorphine significantly increased the production of antibodies by B cells.
Conclusions. Our current studies confirm that macrophages greatly contribute to immunomodulatory effects induced by opioids. Better understanding of mechanisms of immunomodulation by opioids has great importance allowing for evaluation of its beneficial and adverse effects on patient condition.

P.C4.02.05

Immune effects of opioid drugs are modulated by analgesic adjuvants

M. Kozlowski[1,2], *I. Filipczak-Bryniarska*[1], *K. Nazimek*[2], *M. Wasik*[2], *K. Bryniarski*[2];
[1]*Department of Pain Treatment and Palliative Care, Jagiellonian University Medical College, Krakow, Poland,* [2]*Department of Immunology, Jagiellonian University Medical College, Krakow, Poland.*

Introduction. Opioids exert immunomodulatory effects. We have shown that repeated administration of morphine increases cell-mediated allergic response in mice and, as fentanyl, methadone, buprenorphine and oxycodone, enhances the release of proinflammatory cytokines, reactive oxygen intermediates (ROIs) and nitric oxide by macrophages as well as alters their expression of antigen phagocytosis and presentation markers. In contrast to buprenorphine administration, morphine, fentanyl and methadone treatment impairs humoral immunity induced by macrophages. However, little is known about the possible impact of adjuvant drugs on opioid-induced immune effects. Thus, current study aimed at investigating the influence of analgesic adjuvants on morphine-activated immune effects in mice.
Methods. Mice were treated intraperitoneally with morphine, naloxone, amitriptyline, gabapentin and/or venlafaxine for a week. Macrophage-induced humoral immunity was assessed in plaque-forming assay together with measurement of antibody titers in sera of mouse recipients of SRBC-pulsed macrophages from mice treated with different opioids. In addition, macrophage production of ROIs, nitric oxide and cytokines along with expression of surface markers were estimated.
Results. We observed an overall decrease in cytokine, ROIs and nitric oxide production by macrophages from adjuvant-treated mice, with the strongest effect of amitriptyline administration. Further, addition of adjuvants amplified morphine-induced inhibition of humoral immune response activated by macrophages pulsed with SRBC.
Conclusions. Our current results suggest that adjuvants normalize morphine-increased macrophage innate immune activity, which seems to have great importance during sterile inflammation. However, adjuvants additionally impair B cell activation that is reduced by morphine treatment.

P.C4.02.06

Lymph node stromal cells control T follicular helper cells as well as B cell responses directed against a self-antigen

R. Nadafi[1], *E. D. Keuning*[1], *M. N. Erkelens*[1], *A. Bos*[1], *M. van Gool*[1], *A. Breedveld*[1], *R. M. Reijmers*[2], *L. G. M. van Baarsen*[3,4], *R. E Mebius*[1];
[1]*Molecular cell biology and immunology, VU university medical center, Amsterdam, Netherlands,* [2]*Department of hematology, Leiden university medical center, Amsterdam, Netherlands,* [3]*Department of experimental immunology, Academic medical center, University of Amsterdam, Amsterdam, Netherlands,* [4]*Amsterdam Rheumatology & immunology Center (ARC), Academic Medical Center, Amsterdam, Netherlands.*

The hallmark for autoimmunity, such as rheumatoid arthritis, is the production of autoantibodies by B cells against self-antigens. To have an effective germinal center response and antibody production, B cells cooperate with T follicular helper cells (Tfh) to undergo somatic hypermutation and improve the affinity of antigen recognition. Tfh formation and differentiation occurs in the T cell area of the lymph node where a highly organized network of lymph node stromal cells (LNSCs) control and regulate peripheral immunity. Here, we use in-vivo murine lymph node transplantation model and show that LNSCs can clearly repress formation of autoreactive Tfh while maintaining T regulatory cells (Treg) specific for a given self-antigen. Moreover, control of Tfh formation by LNSCs significantly reduced the germinal center B cells response directed against self-antigen. Importantly, inhibition of IL-2 reduced the LNSC-mediated maintenance of Tregs and released the repression of autoreactive Tfh cell formation in-vivo. These findings show that continuous presentation of self-antigens by LNSCs plays a critical role for Treg maintenance while repressing of Tfh and germinal center B cell formation directed against these self-antigens. Ultimately, these findings provide opportunities to modulate humoral immunity at different stages of rheumatoid arthritis.

P.C4.02.07

Intravenous administration of antigen-coupled red blood cells induces suppressor T CD8+ cell recurrent release of exosome-carried miRNA-150 targeting antigen presenting cells in mice

K. Bryniarski[1], ***K. Nazimek***[1], *M. Wasik*[1], *M. Ptak*[1], *W. Ptak*[1], *P. W. Askenase*[2];
[1]*Department of Immunology, Jagiellonian University Medical College, Krakow, Poland,* [2]*Section of Allergy and Clinical Immunology, Yale University School of Medicine, New Haven, United States.*

Introduction. Mouse contact (CHS) and delayed-type hypersensitivity (DTH) responses are suppressed by intravenous administration of syngeneic red blood cells (RBC) coupled with hapten or protein antigen, respectively. Current studies aimed at investigating the mechanism of RBC-induced suppression.
Methodology. CBA/J, C57BL/6 or miRNA-150KO mice were injected intravenously with trinitrophenyl or oxazolone hapten-coupled RBC or with ovalbumin-conjugated RBC and then were either contact sensitized with hapten or intradermally immunized with ovalbumin. In some instances, tolerized mice were either injected intraperitoneally with clodronate liposomes or immunized second time after DTH elicitation. Supernatant of tolerized mouse lymph node and spleen cell culture was filtered and ultracentrifuged (100.000g) and pelleted exosomes were tested for their suppressive activity in adoptively transferred CHS or DTH. In some cases, cells prior to culture were positively or negatively selected according to CD3, CD4 and CD8 expression.
Results. Intravenous injections of hapten or antigen-coupled RBC induced the release of suppressive, miRNA-150-carrying exosomes by T CD8+ cells, that were preliminarily shown to mediate the memory of suppression. Further, suppressive exosomes targeted antigen-presenting macrophages that in turn inhibited CHS or DTH effector T cells, while miRNA-150KO mice failed to produce suppressive exosomes.
Conclusions. Above data showed that antigen-coupled RBC activate suppressor T CD8+ cells, which deliver exosome-carried miRNA-150 that targets antigen-presenting cells to suppress CHS and DTH reaction in mice. Consequently, according to our initial data, we speculate that memory of exosome-mediated suppression develops in mice intravenously administered with RBC. Supported by grant No 2013/11/B/NZ6/02041 from Polish National Scientific Centre (NCN).

P.C4.02.08

Repeated administration of hypotensive drugs and diuretics shifts immunity towards Th2-type in healthy mice

*P. Bryniarski[1], S. Strobel[2], A. Chmielowski[2], M. Michalak[2], K. Bryniarski[1], **K. Nazimek**[1];*
[1]Department of Immunology, Jagiellonian University Medical College, Krakow, Poland, [2]Students' Scientific Society, Department of Immunology, Jagiellonian University Medical College, Krakow, Poland.

Introduction. Nowadays, altered inflammatory reactivity of immune cells, especially those infiltrating perivascular tissues, is associated with pathogenesis of hypertension. However, little is known about possible immunomodulatory effects of clinically relevant hypotensives and diuretics. Therefore, our current studies aimed to investigate the effect of these drugs on immunity in healthy mice.
Methodology. 10-week-old CBA mice were treated intraperitoneally with the following drugs: propranolol, hydrochlorothiazide (10mg/kg), carvedilol, captopril, verapamil, furosemide (5mg/kg), amlodipine (3mg/kg) or olmesartan (1mg/kg) for 7 days. On the third day of drug administration, mice were either sensitized with hapten or intraperitoneally injected with mineral oil. Five days later mice were either challenged with hapten to elicit contact sensitivity (CS), or peritoneal macrophages were collected for assessment of their phenotype, cytokine production or for humoral immunity testing, after pulsing with SRBC.
Results: Amlodipine administration slightly increased generation of oxygen and nitrogen radicals and all drugs' administration caused decreased secretion of pro-inflammatory cytokines and slightly enhanced production of anti-inflammatory cytokines by macrophages, and furosemide increased this effect. SRBC-pulsed macrophages from drug-treated mice more potently activated splenic B cells to release antigen-specific antibodies. Finally, all tested drugs, amlodipine and verapamil especially, at both, the induction and effector phases of CS, suppressed cellular immune response.
Conclusion: Our research findings showed that hypotensives modulate immunity by affecting macrophage function and by polarizing towards Th2-type. Further research should be conducted to examine clinical effect of those observations. Supported by K/DSC/003595 and partly by budget funds for science in 2017-2021 under the "Diamond Grant" program (0168/DIA/2017/46).

P.C4.02.09

Inhibiting squalene synthase increases cellular tolerance to cholesterol-dependent cytolysins

***M. Pospiech**[1], S. Owens[1], D. J. Miller[2], R. K. Allemann[2], I. M. Sheldon[1];*
[1]Swansea University Medical School, Swansea, United Kingdom, [2]Cardiff University School of Chemistry, Cardiff, United Kingdom.

BACKGROUND: During infection bacteria secrete toxins that damage the epithelium of the skin and mucosa. Whilst antibiotics are commonly used to treat infection, another approach is to improve the host tissues to tolerate pathogens. Cholesteroldependent cytolysins are secreted by bacteria and target cholesterol-rich areas of mammalian cell plasma membranes, where they form pores, which leads to cytolysis. The present study aims to inhibit squalene synthase to reduce the biosynthesis of cellular cholesterol and increase cellular tolerance to cholesterol-dependent cytolysins.
METHODS: Novel bisphosphonate compounds were designed to inhibit squalene synthase, and synthesised de novo. The bisphosphonates were screened for their ability to inhibit squalene synthase in a cell-free system using a radiometric assay. Twenty bisphosphonates were then evaluated by treating HeLa cells prior to a challenge with a concentration of the cholesterol-dependent cytolysin, pyolysin, that causes 90% cytolysis. Cytolysis was evaluated using the MTT assay, and pore formation was evaluated by measuring the leakage of cellular potassium ions and LDH protein.
RESULTS: Reference compounds as zaragozic acid and methyl-β-cyclodextrin treatment of cells reduced the cytolysis caused by pyolysin by 95%. Amongst the 20 bisphosphonates, we identified two lead compounds that reduced cytolysis caused by pyolysin in a concentration-dependent manner, with a maximum reduction of cytolysis of 95% and 81%. These lead bisphosphonates also prevented short-term potassium leakage from cells, and reduced longer-term LDH leakage by 92%from cells challenged with pyolysin. In conclusion, inhibition of squalene synthase by bisphosphonates increased the ability of HeLa cells to tolerate a cholesterol dependent cytolysin.

P.C4.02.10

Role of Pbx-regulating-protein 1 (Prep1) in the control of effector and regulatory T cell response in metabolic disorders

***C. Procaccini**[1], D. Faicchia[1], S. Cabaro[2], A. Liotti[2], F. Oriente[3], V. Gigantino[4], F. Blasi[5], P. Formisano[3], F. Beguinot[3], G. Matarese[6];*
[1]Ist. Endocrinologia e Oncologia Sperimentale, Consiglio Nazionale delle Ricerche (IEOS-CNR), Napoli, Italy, [2]URT 'Genomica Funzionale' Istituto di Endocrinologia ed Oncologia Sperimentale, 'G. Salvatore', Consiglio Nazionale delle Ricerche (IEOS-CNR), Napoli, Italy, [3]Dipartimento di Scienze Mediche Traslazionali, Università degli Studi di Napoli, 'Federico II', Napoli, Italy, [4]Unità di Patologia, "Istituto Nazionale Tumori, IRCCS, Fondazione Pascale,", Napoli, Italy, [5]IFOM, FIRC Institute of Molecular Oncology, IFOM-IEO Campus, Milano, Italy, [6]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli "Federico II", Napoli, Italy.

Pbx-regulating-protein 1 *(Prep1)* is an homeodomain transcription factor, which plays an important role in organogenesis and in the regulation of energy homeostasis and metabolism. *Prep1* heterozygous mice (*Prep1*$^{i/+}$), expressing 55-57% of protein, display increased sensitivity to insulin and are protected from diabetes. As accumulating evidence has shown that metabolic disorders such as obesity and type 2 diabetes are associated with immune system dysfunction, in this study we characterized the role of *Prep1* in the modulation of immune response, associated with protection from metabolic disorders. *Prep1* deficiency significantly inhibited T cell proliferation, by decreasing their activation and secretion of pro-inflammatory cytokines. These effects were associated with an impaired activation of the mammalian-target of rapamycin (mTOR) pathway, cell growth arrest and an altered bioenergetic profiling of CD4$^+$ T cells. On the contrary, CD4+CD25^{hi}Foxp3$^+$ regulatory T cells (Treg) from *Prep1*$^{i/+}$mice displayed a higher ex vivo proliferative capacity and increased suppressive activity, which determined protection of these mice from metabolic alterations, hepatic inflammation and steatohepatitis, induced by high fat diet (HFD). These data unmask a previously unknown role of *Prep1* in the regulation of T cell functions, suggesting that Prep1 might represent a novel platform for potential therapeutic manipulation of immune system functions to control immune-mediate metabolic disorders.

P.C4.02.11

Identification of immune tolerance-related aberrant epigenetic hubs in T cells from multiple sclerosis patients

*D. Avancini[1], V. Martinelli[2], C. Farina[2], S. Gregori[1], **F. R. Santoni de Sio**[1];*
[1]San Raffaele Telethon Institute for Gene Therapy (SR-TIGET), San Raffaele Scientific Institute IRCCS, Milan, Italy, [2]Institute for Experimental neurology (INSPE), San Raffaele Scientific Institute IRCCS, Milan, Italy.

The molecular mechanisms underlying the breakage of immune tolerance triggering multiple sclerosis (MS) autoimmune manifestations remain mainly unclear. Recently, deregulation in epigenetic control has been associated with immune cell alterations and MS-related autoimmunity. We have studied epigenome and transcriptome in CD4+ T cells from peripheral blood of relapsing-remitting MS patients and matched healthy controls (HC) to identify aberrant epigenetic hubs (nodes of aberrantly activated regulatory elements and target genes). We identified by ChiP-seq analysis a number of Differentially Active Regulatory Elements (DARE), most of which active in HC and less/not active in MS cells. We defined DARE-target genes by intersecting the DARE list with public 3C-based datasets. DARE-target genes resulted over-represented among genes up-regulated in the RNA-seq analysis of HC and enriched in autoimmune/inflammatory disease gene ontology classes, confirming the differential activity of identified DARE in MS and HC T cells and suggesting their functional role in MS autoimmune manifestations.
To assess the molecular network controlling the activity of MS-DARE, we in silico mapped transcription factor (TF) binding sites and found some interacting master TF enriched for binding at DARE and DARE-target gene promoters, suggesting that a core of TF might control the activity of MS-DARE. Our data suggest aberrant control of regulatory elements as player in T cell alterations in MS. Current studies aimed at validating chromatin data by ATAC-seq and evaluating the role of identified altered epigenetic hubs in T cell function will assess the pathogenic role of identified alteration in MS and in autoimmune diseases in general.

P.C4.02.12

Regulatory T cell numbers in inflamed skin controlled by ALK3 signaling in dendritic cells

***T. Sconocchia**[1], M. Hochgerner[2], I. Borek[1], E. Schwarzenberger[1], C. Tam-Amersdorfer[1], H. Strobl[1];*
[1]Otto Loewi Research Center, Chair of Immunology and Pathophysiology, Graz, Austria, [2]Institute of Cancer Research, Medical University of Vienna, Vienna, Austria.

Bone morphogenetic protein 7 (BMP7) is expressed at aberrant high levels by keratinocytes during skin inflammation and induces inflammatory-type Langerhans cell (LC) differentiation. Regulatory T cell (Treg) are increased in inflamed epidermis and LCs are known to stimulate Tregs. Thus, we asked whether BMP signaling in LCs may promote Treg generation. Human LCs were generated from CD34$^+$ cells in response to either BMP7 or TGF-β1, to generate LCs resembling inflammatory LCs or steady-state-like LCs, respectively. LCs were cultured with allogeneic CFSE-labeled naïve CD4$^+$ T cells. BMP7-LCs displayed an enhanced T cell stimulatory capacity compared to TGF-β1-LCs. Also, the extent of Treg content utilizing BMP7-LCs, was significantly higher than that induced by TGF-β1-LCs. In addition, Tregs derived from BMP7-LCs were significantly more immunosuppressive than that derived from TGF-β1-LCs. *In vivo* validation was carried out by measuring FoxP3$^+$ cell frequencies in DC-specific ALK3/BMPR1a knockout mice in an imiquimod induced model of skin inflammation. ALK3 deficiency was associated with fewer FoxP3$^+$ cells compared to wild type mice. Subsequently, molecules that interfere with LC-mediated Treg induction were evaluated. An interfering ALK3 fusion protein specifically reduced Treg generation by BMP7-LCs. Vice versa, BMP7 directly promoted Treg differentiation in an APC-free system from naïve T cells *in vitro*. These data demonstrate that BMP/ALK3 signaling in inflamed skin enhances Treg numbers at least partially via modulation of DCs.

P.C4.02.13

Investigation of the molecular mechanisms that affect regulatory T cell function in patients with active systemic lupus erythematosus

*C. Albany[1], Z. Catak[1], D. McCluskey[1], G. Giganti[1], L. Nel[2], G. A. Povoleri[1], M. Catapano[1], M. Robson[1], J. Spencer[1], D. D'Cruz[2], R. I. Lechler[1], G. Lombardi[1], **C. Scotta**[1];*
[1]King's College London, London, United Kingdom, [2]Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom.

Systemic lupus erythematosus (SLE) is a multisystem, chronic, debilitating and frequently-relapsing condition. Although anti-inflammatory treatments are available, none is curative and relapses remain common. There is a critical requirement for effective therapies that obviate the need for immunosuppression and restore self-tolerance. Regulatory T cells (Tregs) in healthy individuals prevent the development of autoimmune diseases; however, in SLE-patients Tregs are numerically deficient and/or functionally impaired. *Ex vivo* expansion of SLE-Tregs along with the repair of their function to obtain a clinical product for cell-therapy is an attractive and novel option for re-establishing self-tolerance. Functional, phenotypic and genetic data were collected from SLE-Tregs either freshly isolated or cultured in the presence of rapamycin. Results showed that although SLE-Tregs resemble the typical phenotype of healthy-Tregs, their suppressive capacity is impaired during active disease. The analysis of Treg sub-populations revealed differences in cell-distribution during the relapsing and remitting course of the disease that correlate with the breakdown of self-tolerance. However, the *ex vivo* expansion of Tregs from SLE-patients in the presence of rapamycin fully restored their function. The analysis of molecular data from SLE-Tregs identified a list of differentially expressed genes/molecular pathways that describes both the signature of dysfunctional Tregs and the 'repairing effect' of rapamycin. Altogether our data indicate that the functional defect of Tregs in SLE can be corrected along with their expansion in a therapeutic cell-product. Our findings suggest a new approach to use autologous Tregs in a programme of adoptive-cell-therapy for the treatment of SLE.

P.C4.02.14

Immune inhibitory CD200-Receptor potentiates type I immunity during inflammation

***M. Van der Vlist**[1], M. Ramos[1], L. van den Hoogen[2], S. Hiddingh[2], M. van der Kroef[2], R. Lebbink[2], T. Radstake[2], L. Meyaard[1];*
[1]UMC Utrecht; Oncode Institute, Utrecht, Netherlands, [2]UMC Utrecht, Utrecht, Netherlands.

Immune responses are tightly regulated to allow pathogen clearance but prevent autoimmunity. Systemic Lupus Erythematosus (SLE) is an autoimmune disease that predominately affects women (9:1) and arises from dysregulated Toll-like receptor (TLR) 7 signaling and aberrant type I interferon production. The CD200 Receptor 1 (CD200R) is an inhibitory immune receptor that limits TLR7-induced type I interferon responses especially in females. Therefore, we hypothesized that CD200R-mediated inhibition is absent in SLE patients. Surprisingly, we discovered that in PBMC from SLE patients TLR-induced cytokine production was not suppressed by CD200R but was instead potentiated by it. CD200R signaling also potentiated TLR-induced cytokine production in healthy control (HC) PBMCs treated with IFNα. We found that in the absence of inflammation, CD200R inhibited Akt activation through Dok2 and Erk/rpS6 activation through RasGAP, resulting in suppression of cytokine production. In contrast, SLE PBMC or HC PBMC treated with IFNα had decreased RasGAP to levels that were insufficient for CD200R to inhibit Erk/rpS6. Furthermore, CD200R retained its ability to inhibit Akt via Dok2, which normally provides negative feedback on type I cytokines. Thereby CD200R potentiated type I cytokine production. We conclude that IFNα negatively regulates CD200Rs ability to dampen inflammatory response through RasGAP, while leaving intact its ability inhibit Akt. Taken together, we demonstrate that the signal transduction machinery of the immune-inhibitory receptor CD200R is responsive to type I IFNs thereby allowing CD200R to switch from inhibitory to potentiating depending on the inflammatory environment.

P.C4.02.15

Immunomodulatory properties of cellulose nanocrystals depend on their functionalization

***M. Vasiljevic**[1], M. Bekic[2], B. Joksimovic[1], D. Mihajlovic[3], M. Milanovic[3], I. Majstorovic[3], D. Vucevic[3], S. Tomic[2], M. Colic[2,1,3];*
[1]Medical Faculty Foca, University of East Sarajevo, Foca, Bosnia and Herzegovina, [2]Institute for the Application of Nuclear Energy, Belgrade, Serbia, [3]Medical Faculty of the Military Medical Academy, University of Defence, Belgrade, Serbia.

Cellulose nanocrystals (CNC) are attractive nanomaterials with large surface area suitable for development of drug delivery and diagnostic systems. However, the biocompatibility and immunomodulatory properties of CNC have not been studied so far, especially in relation to CNC functionalization. Here we used wood-based native (n)CNC, as precursors for TEMPO-oxidized (o)CNC and phosphonated (p)CNC, to assess their toxicity and immunomodulatory potential on human peripheral blood mononuclear cells (PBMC) and monocyte-derived dendritic cells (MoDC). We found that non-toxic concentrations (<400 μg/ml) of nCNC and oCNC impaired the proliferation and IL-2 production by phytohemaglutinin-stimulated PBMC, whereas pCNC had no significant effects. According to CD14/CD1a expression analysis, oCNC displayed the strongest inhibitory effect on MoDC differentiation, followed by nCNC and pCNC, respectively. These results correlated with the weakest maturation capacity of oCNC-treated MoDC induced by LPS/IFN-γ. Additionally, nCNC- and oCNC-treated MoDC expressed higher levels of PD1-L, TGF-β and ILT-4 compared to control MoDC, whereas pCNC-treated MoDC showed no such property. The capacity of MoDC to produce high levels of IL-12p70, IL-1β, IL-23, and low levels of IL-10, were impaired by nCNC and oCNC, but not by pCNC. In line with this, nCNC- and oCNC-treated MoDC displayed an increased capacity to induce alloreactive Th2 cells and TGF-β-producing CD4+CD25^{hi}FoxP3+ Treg cells, and a decreased capacity to induce IFN-γ-producing Th1 cells in co-culture. Cumulatively, these results suggest that CNC may induce tolerogenic properties in MoDC, whereas phosphonation of CNC prevents such effects, thus restoring the immunogenic potential of MoDC.

P.C4.02.16

Investigation of EZH2 as an epigenetic modulator of FoxP3 expression in regulatory T-cells in the light of immune tolerance impairment

***A. Velichkov**[1], R. Susurkova[1], A. Mihova[2,3], M. Guenova[2], I. Antonova[4], D. Staneva[5], M. Georgieva[5], G. Nikolov[4], G. Miloshev[5], V. Terzieva[1,3];*
[1]Institute of Biology and Immunology of Reproduction, BAS, Sofia, Bulgaria, [2]National Specialised Hospital for Active Treatment of Haematological Diseases, Sofia, Bulgaria, [3]University Hospital "Lozenetz", Sofia, Bulgaria, [4]Medical Center "Reprobiomed", Sofia, Bulgaria, [5]Laboratory of Molecular Genetics, Institute of Molecular Biology "R. Tsanev", Bulgarian Academy of Sciences, Sofia, Bulgaria.

Introduction: Regulatory T-cells (Tregs) represent the effective arm of immune tolerance. Their function is tightly regulated and its impairment is associated with recurrent pregnancy loss (RPL), autoimmune and allergic reactions. Our aim is to evaluate EZH2, an epigenetic modulator of FoxP3 expression by the model of recurrent pregnancy loss.
Materials and Methods: PBMCs from healthy controls (HCs) with no RPL: women with successful pregnancy (n=24; 23-45 years) and without pregnancy (n=10; 26-38 years) and patients with RPL (n=18; 25-44 years) were stained with anti-CD3/CD4/CD45RA/CD25/FOXP3 antibodies. FoxP3 and EZH2 expression were determined by PrimeFlow™ assay and qRT-PCR in non-stimulated or stimulated with progressively increased progesterone concentrations. The FACS-analysis was done using FlowJo V10 and the Statistical analysis by GraphPad Prism7.
Results: The overall analysis showed lower CD45RA+FOXP3+nTregs percentage in patients compared to the healthy groups ($p<0.05$). The highest proportion of CD25+nTregs was found in the group of women w/o pregnancy ($p<0.05$). Conversely, the proportion of CD45RA+FOXP-CD25-T-cells dominated in the group of patients ($p<0.05$). The percentage of EZH2mRNA+ cells in non-Tregs and Tregs was greater in HCs ($p<0.05$) and showed progesterone-dependent variations.
Conclusion: Our results show that the pregnancy impacts mainly nCD4+ population. Epigenetic modifications facilitated by the hormonal milieu might be associated with variations in FoxP3 expression. Considering that EZH2 and FOXP3 are indispensable for the fate of Treg, further investigations are inline to clarify the precise regulation mechanisms in Tregs.
Acknowledgements: This work was supported by Grant DN03/4-2016 of National Science Fund, Ministry of Education and Science, Republic of Bulgaria.

P.C4.02.17

Genome-wide methylation analysis of regulatory and conventional T cells

***A. Salumets**[1], H. Peterson[1], P. Peterson[2];*
[1]Institute of Computer Science, Tartu, Estonia, [2]Institute of Biomedicine and Translational Medicine, Tartu, Estonia.

Regulatory T cells (Tregs) represent a subpopulation of T cells that are specialised in immune suppression and maintenance of immune tolerance. They have a crucial role in the prevention of autoimmunity and their failure leads to development of autoimmune diseases. We compared the Tregs to their conventional counterparts (Tconvs) that serve an opposing role - activation of immune system. The aim of our study was threefold: (1) to examine differentially methylated positions (DMPs) and regions (DMRs) between Tregs and Tconvs; (2) find DMPs and DMRs between Tregs from healthy controls and Graves' patients; and (3) find which DMPs and DMRs from the previous step are characteristic to Tregs. To meet the objectives, we performed a genome-wide methylation analysis with Infinium Human Methylation EPIC BeadChips. Firstly, we focused on CpG sites that were differentially methylated between Tregs and Tconvs (6 healthy controls). As a result, we found nearly 19,000 DMPs and 630 DMRs (FDR<0.05). Secondly, we investigated differences between Tregs from healthy controls (10 individuals) and Graves' patients (11 individuals) which resulted in 19 DMPs (FDR<0.05). Our analysis indicated no overlap between those two sets of DMPs. However, we observed differences between methylation value distribution of Tconvs and Tregs where Tconvs had more extreme methylation values i.e. they had more CpGs with either low or high methylation level.

P.C4.03 Manipulation of tolerance - Part 3

P.C4.03.01
Expression of PHD2 regulates regulatory T cell capacity to control Th1-like inflammatory responses

***Y. Ajouaou**, H. Hussein, S. Denanglaire, F. Andris, M. Moser, O. Leo;*
Université Libre de Bruxelles, Gosselies, Belgium.

Inflammation is often associated to a hypoxic state imposing a metabolic constraint on inflammatory cells. The protein HIF1α plays an important role in cells hypoxia adaptation, and its stability is regulated by the oxygen sensor prolyl-hydroxylase 2 (PHD2) in numerous cell types including T lymphocytes. The impact of hypoxia on immune cells, in particular on regulatory T cell (Treg) function, has not been fully elucidated. The purpose of our study is to evaluate the role of the PHD2-HIF1α axis in the regulation of homeostasis and function of Tregs.
We demonstrate in this work that selective ablation of PHD2 expression in Tregs ($PHD2^{\Delta Treg}$ mice) leads to a spontaneous intestinal inflammatory syndrome, as evidenced by the development of a rectal prolapse and elevated expression of IFNγ and IL-10 in the mesenteric lymph nodes and spleen. PHD2 deficiency in Tregs leads to an increased number of activated CD4 and CD8 conventional T cells expressing an effector-like phenotype ($CD44^{hi}CD62L^{low}$). Concomitantly, the expression of innate-type cytokines such as IL1-β, IL-12p40, IL-12p35 and TNF-α is found to be elevated in peripheral (gut) tissues. Finally, $PHD2^{\Delta Treg}$mice display an enhanced sensitivity to DSS-induced colitis and to experimental autoimmune encephalomyelitis (EAE), suggesting that PHD2-deficient Tregs do not efficiently control inflammatory response *in vivo*. The mechanisms whereby PHD2 controls Treg activity is presently under investigation, in particular through the development of *in vitro* models of immune suppression. We hope that our study will contribute to a better understanding of the role of oxygen-sensing pathways in the regulation of inflammatory responses.

P.C4.03.02
Short term cold acclimation enhances human Treg induction

***M. Becker**[1,2], I. Serr[1,2], L. Mengel[3], H. Hauner[3,4], M. H. Tschöp[2,5,6], C. Daniel[1,2];*
[1]Institute of Diabetes Research, Group Immune Tolerance in Diabetes, Helmholtz Diabetes Center at Helmholtz Zentrum München, German Research Center for Environmental Health, Munich, Germany, [2]German Center for Diabetes Research (DZD), Munich, Germany, [3]ZIEL-Institute for Food & Health, Else Kröner-Fresenius Zentrum für Ernährungsmedizin, Technische Universität München, Freising-Weihenstephan, Germany, [4]Institute for Nutritional Medicine, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany, [5]Institute for Diabetes and Obesity, Helmholtz Diabetes Center, Helmholtz Zentrum München, Munich, Germany, [6]Division of Metabolic Diseases, Technical University of Munich, Munich, Germany.

$Foxp3^+$regulatory T-cells (Tregs) function as critical immune regulators, thereby controlling tissue-specific differentiation, homeostasis and local inflammation. We recently found that murine Tregs represent key components of the molecular interface connecting adipose tissue (AT) function with environmental cold or low-dose beta3-adrenergic stimulation. Specifically, by loss- and gain-of-function experiments, including Treg depletion and transfers in vivo, we identified a T-cell-specific Stat6/Pten signaling axis that links cold exposure or beta3-adrenergic stimuli with Treg activity and AT function. However, the translational relevance of these findings for human Treg induction in response to beta3-adrenergic stimulation or cold remains currently unknown. Here, we show that beta3-adrenergic stimulation using Mirabegron (Mira) induces human Tregs in a preclinical setting of humanized NSG mice (human $CD3^+CD4^+CD127^{low}CD25^{high}Foxp3^+$Tregs [% of $CD4^+$T cells]: control: 1.9±0.5 vs. +Mira: 4.9±1.0; p=0.0319) accompanied by increased Treg induction potential from naïve $CD4^+$T cells in vitro. Moreover, human $CD4^+$T cell analyses of subcutaneous AT biopsies after an acute cold stimulus of 2 hours to healthy subjects provide first evidence for an increase in local $CD3^+CD4^+CD127^{low}CD25^{high}Foxp3^+$Tregs (Tregs [% of $CD3^+CD4^+$]; t0=1.54±0.34% vs. t2=2.31±0.98%). Of note, short-term human cold acclimation in vivo also enhanced human Treg induction potential from naïve $CD4^+$T cells in peripheral blood ($CD3^+CD4^+CD127^{low}CD25^{high}Foxp3^+$Tregs [% of t0]; t0=100.0±7.5% vs. t2=137.1±25.9%; p=0.0030). These findings support the concept that cold exposure or beta3-adrenergic stimulation can exert pro-tolerogenic functions on human $CD4^+$T cells. Further mechanistic analyses are required to dissect molecular underpinnings of human $Foxp3^+$Treg induction in response to cold or beta3-adrenergic stimulation in health and metabolic disease.

P.C4.03.03
Salt modulates cellular metabolism of regulatory T cells

***B. F. Côrte-Real**[1], O. Matveeva-Kolm[1], I. Hamad[1], A. Geuzens[1], L. Dubois[2], M. Kleinewietfeld[1];*
[1]VIB Laboratory for Translational Immunomodulation, VIB Center for Inflammation Research (IRC), Hasselt, Belgium, [2]Department of Radiation Oncology (Maastro), GROW - School for Oncology and Developmental Biology, Maastricht, Netherlands.

High salt intake has been associated with shifts in the immune cell balance, mainly by promoting proliferation and activity of pro-inflammatory cells, such as T helper 17 (Th17) and M1 macrophages, and by impairing the functions of anti-inflammatory cells such as regulatory T cells (Tregs) and M2 macrophages. However, the precise molecular mechanisms that lead to this phenotype are still unknown. The role of metabolic regulation in shaping immune responses has gained increasing attention in recent years. Cellular metabolism is a vital process, which is essential for growth, survival and proliferation of every cell type, and can be greatly influenced by environmental factors such as diet. A recent study has shown that high-salt leads to metabolic changes in M2 macrophages by decreasing their mitochondrial oxidative phosphorylation (OXPHOS) and glycolysis necessary for their activation. Therefore, we analyzed the effect of high-salt on human Treg metabolism. Our results on human Tregs show a similar trend induced by high-salt as the ones observed in macrophages. This changes might be linked to the loss of suppressive function of Tregs upon high-salt challenge. M.K. was supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (640116), by an SALK grant from the government of Flanders, Belgium, and by an Odysseus grant from the Research Foundation Flanders, Belgium (FWO).

P.C4.03.04
Treg profile associated with long-term immunosuppressed transplanted patients with cancer

***L. Daniel**[1,2], L. Dufour[3], A. Robin[1,3], A. Herbelin[1,2], J. Gombert[1,2,3], A. Thierry[1,3];*
[1]Inserm U1082, Poitiers, France, [2]Université de Poitiers, Poitiers, France, [3]CHU de Poitiers, Poitiers, France.

Background: The long-term immunosuppressive therapy required to maintain host tolerance of a transplanted organ contributes to an increased risk for malignancy in organ transplant recipients. A variety of factors, including the intensity and duration of immunosuppression, can influence the likelihood for the development of cancer in these patients. Here, we focused on the regulatory CD4(+)CD25(+)FoxP3(+) T cells (Treg) as possible targets for the immunosuppressive treatments, and explored their profile associated with cancer.
Methods: We analyzed by flow cytometry blood samples from 60 renal transplant recipients for more than 10 years, without signs of rejection, treated with minimized immunosuppression: azathioprine+steroids or anticalcineurin monotherapy.
Results: We observed an increase in Treg frequency and memory Treg CD45RA(-)FoxP3(hi) frequency in patients with a history of cancer, compared to patients without cancer. We also observed an increase of CTLA4 expression in Treg from anticalcineurin-treated patients with a history of cancer, compared to patients without cancer and healthy controls. Lastly, we observed an increase of CD4(+)FoxP3(+)Helios(+) Treg with a stable immunosuppressive phenotype in patients treated with azathioprine+steroids and a history of cancer, whereas the frequency of these cells decreased in the other groups of patients compared to healthy controls.
Conclusion: The proportion and functional status of Treg appeared to vary according to the type of treatment and background of cancers in renal transplant patients with long-term minimized immunosuppression. Further assays evaluating donor-specific responses of recipient Treg in our cohort would promote a better understanding of the mechanisms underlying operational tolerance in transplantation.

P.C4.03.05
Identification of a tolerogenic Factor V peptide and its potential role in Factor VIII tolerance induction

***A. S. De Groot**[1,2], A. Rosenberg[3], S. Lelias[1], P. Hindocha[1], F. Terry[1], B. Roberts[1], W. Martin[1], E. Guillen[1];*
[1]EpiVax, Inc., Providence, United States, [2]University of Rhode Island, Providence, United States, [3]Center for Drug Evaluation and Research, FDA, White Oak, United States.

Inhibitory Anti-Drug Antibody (ADA) responses interfere with Factor VIII replacement efficacy in 25-30% of Hemophilia A (HA) cases, greatly increasing patient morbidity and treatment costs. As an extension of previous work on tolerance-inducing peptides in IgG (Tregitopes), we investigated whether there were peptides in other ubiquitous serum proteins that could have high homology to peptides found in Factor VIII. We hypothesized that tolerance to cross-conserved peptides in other prevalent proteins might explain why anti-FVIII antibodies fail to develop in some severely FVIII-deficient HA patients. Using advanced computational modeling tools, we discovered in Factor V a potent regulatory peptide (FVP4) with an immunologic profile (HLA and TCR binding) that is homologous to a non-identical peptide in FVIII. We postulated that treatment with a FVP4-peptide containing biologic may be able to invoke tolerance to Factor VIII in patients who have anti-FVIII antibodies. In an ex vivo assay using human PBMC, we found that the CD4 and CD8 effector recall response was strongly inhibited by FVP4. However, other Factor V peptides with similar HLA-binding properties did not suppress the response. The peptide homologue to FVP4 found in Factor VIII shows similar inhibitory activity on activated CD4 T cells. Using this assay, we are determining the evolution of markers on regulatory T cells, Antigen Presenting Cells, and effector T cells as well as the cytokine secretion profile over time in order to identify specific parameters associated with cell populations and soluble factors mediating immune suppression in response to FVP4.

P.C4.03.06

Functional defect of regulatory T cells in Anti-Neutrophil Cytoplasmic Autoantibody associated vasculitis is associated with overexpressionof microRNA-142-3p

G. Dekkema[1], *T. Bijma*[2], *W. H. Abdulahad*[3], *P. G. Jellema*[1], *A. Van Den Berg*[1], *B. Kroesen*[4], *C. A. Stegeman*[2], *P. Heeringa*[1], *J. Sanders*[2];
[1]*Department of Pathology and Medical Biology, University Medical Center Gron, Groningen, Netherlands,* [2]*Department of Internal Medicine, division of Nephrology, University Medical Center Gron, Groningen, Netherlands,* [3]*Department of Rheumatology and Clincial Immunology, University Medical Center Gron, Groningen, Netherlands,* [4]*Department of Clinical Immunology, University Medical Center Gron, Groningen, Netherlands.*

Introduction: Circulating regulatory T cells (Tregs) in anti-neutrophil cytoplasmic antibody associated vasculitis are frequently functionally deficient. The mechanism behind their impaired function is however unknown. Here, we hypothesized that the dysfunctionality of Tregs in AAV is due to altered microRNA (miR) expression in these cells.
Methods/results: Tregs (CD4+CD45RO+CD25+CD127-) of healthy controls (HC) and AAV patients in remission without treatment (AAV-REM) were FACS-sorted, and total RNA was isolated. Samples from 8 HCs and 8 AAV-REMs were subjected to miRNA microarray analysis. Nineteen miRNAs were differently expressed, and in an independent validation cohort, miR-142-3p was confirmed to be significantly upregulated (2-fold, p=0.03) in Tregs of AAV-REM patients (n=23). In vitro transient overexpression of miR-142-3p using a mimic-miR-142-3p, showed that the suppressive capacity of Tregs was significantly inhibited upon overexpression (1.9 fold reduction, p=0.02), and miR-142-3p levels tended to negatively correlate to the suppressive capacity of Tregs (p=0.06, rho=-0.591). A database and literature search identified adenylyl cyclase-9 (AC9) as validated target for miR142-3p. mRNA levels of AC9 (3.8-fold) tended to be lower in AAV-REM Tregs. In addition, cyclic AMP (cAMP) levels in Tregs, partly produced by AC9, were measured after aCD3/CD28 stimulation. After 48h stimulation, cAMP levels were significantly lower in AAV-REM Tregs (1.7 fold, p=0.003). Moreover, overexpression of miR-142-3p also significantly lowered the cAMP production.
Conclusion: Increased expression of miR-142-3p in Tregs of AAV-REM patients may induce their functional impairment by targeting the AC9/cAMP mediated suppression.

P.C4.03.07

In vivo screening of novel fusion proteins targeting CD28 and PD1 pathways inhibiting immune responses and promoting long-term transgene expression in the context of muscle gene therapy

L. Dupaty[1], *M. Demeules*[1], *G. Riou*[1], *L. Jean*[1], *A. Salvetti*[2], *O. Boyer*[1], *S. Adriouch*[1];
[1]*Normandie Univ, UNIROUEN, INSERM, U1234, Physiopathologie, Autoimmunité, maladies Neuromusculaires et THErapies Régénératrices (PANTHER), Rouen, France,* [2]*Cancer Research Center of Lyon (CRCL), INSERM U1052, CNRS UMR5206, Lyon, France.*

In vivo gene transfer mediated by adeno-associated viral (AAV) vectors has become a feasible therapeutic strategy in human. However, immune responses against the therapeutic gene products (TgP) represent a major concern as this method widened to a spectrum of pathologies and individuals. One remaining challenge is to induce immunological tolerance towards the TgP.
For that, we implemented a stringent animal model and evaluated a panel of 12 novel fusion proteins derived from CTLA-4 and/or PDL1 with the aim to manipulate these major immunoregulatory axes and to induce long-term tolerance. To directly screen in vivo the immunoregulatory properties of our selected protein candidates, we used AAV vectors to produce them in vivo and co-injected them together with an AAV vector coding for the strongly immunogenic Ovalbumin TgP. This screening strategy allowed the identification of 2 immunoregulatory candidates, PD-L1^{n1} and PD-L1^{n2}, that significantly inhibit cellular and antibody responses against the TgP and, remarkably, improved Ova persistence *in vivo*. Interestingly, and in contrast to CTLA-4/Fc, these molecules preserve the Treg compartment and are associated with active immunoregulation rather than inhibition of lymphocytes priming.
Finally, based on our results, we implemented sequential strategy relying on a single injection of CTLA-4/Fc, to inhibit the priming of initial immune response, subsequently followed by the injections of our selected PD-L1^{n1} or PD-L1^{n2} immunoregulatory proteins. This strategy may be of interest in gene therapy as well in transplantation where long-term tolerization remains a major challenge.

P.C4.03.08

A novel approach for the isolation of medullary thymic epithelial cells from murine thymi improves purity and cell recovery

R. Engelmann[1], *D. Dohr*[2], *B. Müller-Hilke*[1];
[1]*Institute of Immunology & Core Facility for Cell Sorting and Cell Analysis, Rostock, Germany,* [2]*Institute of Immunology, Rostock, Germany.*

Objective: Medullary thymic epithelial cells (mTEC) play a central role in the removal of T cells specific for tissue-restricted antigens and thus prevent disastrous autoimmunity. Thus methods to efficiently isolate these cells are warranted. **Methods:** Excised murine thymi were digested with a standard dispase/collagenase/DNase mixture. Thereafter, mTEC were magnetically enriched using UEA-1 microbeads and CD45+ thymocytes were subsequently depleted by cell straining. This novel method was compared to the broadly used enrichment by percoll density gradient centrifugation followed by flow cytometry cell sorting. **Results:** The usage of 2µl UEA-1 beads per 1x10^8 cells for magnetic enrichment was superior to the percoll method in terms of the percentage of enriched mTEC (22% versus 1.2% on average) and the number of isolated mTEC per thymus (2x10^5 versus 5x10^4). The viability after both procedures was comparable. Subsequent depletion of CD45+ cells resulted in an mTEC purity of 76% compared to 87% after flow cytometric cell sorting. However, the recovery rate of cells proved to be significantly higher after cell straining as compared to flow cytometric cell sorting. **Conclusions:** The combination of magnetic enrichment of UEA-1+ cells with subsequent depletion of CD45+ cells via cell straining results in increased yields of mTEC as compared to the current gold standard method.

P.C4.03.09

Prostaglanin-E2 potentiate the suppressive functions of human GM-CSF/IL-6-induced myeloid-derived suppressor cells *in vitro*

B. Joksimovic[1], *M. Bekic*[2], *M. Vasiljevic*[1], *M. Milanovic*[3], *D. Vucevic*[3], *S. Tomic*[2], *M. Colic*[2,1,3];
[1]*Medical Faculty Foca, University of East Sarajevo, Foca, Bosnia and Herzegovina,* [2]*Institute for the Application of Nuclear Energy, Belgrade, Serbia,* [3]*Medical Faculty of the Military Medical Academy, University of Defence, Belgrade, Serbia.*

Myeloid derived suppressor cells (MDSC), discovered as one of the major factors driving tumor progression, are also involved in beneficial effects during the course of autoimmune diseases, but their role is not fully understood. The protocols for differentiation of human monocyte-derived MDSC *in vitro* have not been fully established, especially considering the immense role of prostaglandin (PG)-E2 in the induction of MDSC *in vivo*. Here we found that GM-CSF and IL-6 were required for the induction of CD33$^+$HLA-DR^{low}CD14$^+$CD209$^-$ MDSC *in vitro* that failed to up-regulate/express co-stimulators and IL-12p70 upon LPS/IFN-γ stimulus. However, the addition of PG-E2 to GM-CSF/IL-6 cocktail up-regulated additionally CD14 and CCR7, and down-regulated the expression of CD1a, HLA-DR, CD209, CD16, CD11c, CD11b on these cells. PG-E2-induced MDSC expressed higher levels of CD39, PD1L, ILT-3 and ILT-4, and produced higher amounts of TGF-β and IL-27, and lower amounts of IL-1β, IL-10, IL-23 and TNF-α after LPS/IFN-γ stimulation, compared to GM-CSF/IL-6-induced MDSC. Alloreactive T cells co-cultured with PG-E2-induced MDSC contained more IL-4-producing CD4$^+$GATA-3$^+$ T cells, and less IL-17-producing CD4$^+$ RORγt$^+$ T cells, as well as IFN-γ-producing CD8+ T cells, but not CD4+IFN-γ+ T cells. Interestingly, PG-E2-induced MDSC generated a smaller percentage of alloreactive CD4+CD25^{hi}FoxP3+TGF-β Tregs, but increased significantly the percentage of CD4+IL-10+FoxP3-IL-4- Tr-1 and CD8+IL10+ T cells, compared to GM-CSF/IL-6-induced MDSC. Therefore, PG-E2 potentiate the suppressive phenotype and functions of GM-CSF/IL-6-induced MDSC, and change the mechanisms involved in Treg induction, which could be important in development of therapeutic strategies focused on MDSC-related effects in tumors and autoimmune diseases.

P.C4.03.10

Post-translational modified protein antibodies in Rheumatoid arthritis: searching for the eye of the storm

A. S. B. Kampstra, *J. Dekkers, A. M. Dorjee, M. Volkov, L. Hafkenscheid, A. Kempers, M. A. van Delft, T. M. Huizinga, D. van der Woude, R. E. Toes;
Leiden University Medical Center, Leiden, Netherlands.*

Autoantibodies against post-translationally modified proteins (Anti-modified Protein Antibodies or AMPA) are a hallmark of Rheumatoid Arthritis (RA). A variety of AMPAs against different protein modifications, such as citrullinated proteins, carbamylated proteins and acetylated proteins have now been described in RA. Since these antibodies are far less frequently found in health or patients with other auto-immune diseases, a shared 'developmental' basis is suggested. At present, the origin or mutual relationship of AMPAs is poorly understood. Here, we aimed to study the origin of AMPA-responses by postulating that the AMPA-response shares a common "background" that evolves over time into different classes of AMPAs. Immunisation of mice with carbamylated proteins not only induced an antibody response recognizing carbamylated proteins, but also acetylated proteins. Similarly, also immunization with acetylated proteins led to the formation of (autoreactive) AMPAs against other modifications as well. Analysis of antibodies purified with citrullinated antigens (ACPA) from blood of RA-patients antibodies revealed that ACPA, besides citrulline-reactivity, can also display reactivity to acetylated and carbamylated peptides. Similarly, purified anti-carbamylated protein antibodies showed cross-reactivity against all three post-translational modifications. Our data show that different AMPA-responses can evolve from exposure to only one type of modified protein. These findings indicate that the different AMPA-responses originate from a common "precursor" B cell response that diversifies into multiple distinct AMPA-responses over time and explain the presence of multiple AMPAs in RA, one of the hallmarks of disease.

P.C4.03.11

Modulating the production of active TGF-b1 by Tregs with antibodies against GARP/latent TGF-b1 complexes

F. Lambert[1], *B. Van Der Woning*[2], *M. Saunders*[2], *S. Lucas*[1];
[1]*de Duve Institute, Brussels, Belgium,* [2]*arGEN-X BVBA, Gent, Belgium.*

Regulatory T cells (Tregs) exert contact-dependent inhibition of immune cells through the production of active TGF-β1. This immunosuppressive cytokine is secreted by all immune cells as a latent and inactive form, in which the mature cytokine is associated to the Latency Associated Peptide (LAP), that precludes the interaction of the mature cytokine with its receptor. To exercise its activity, mature TGF-β1 must be released from the LAP, a process referred to as « TGF-β1 activation ». Tregs activate latent TGF-β1 via a mechanism that requires a transmembrane protein called GARP, which binds latent TGF-β1 by forming disulfide bonds with LAP. We wish to derive monoclonal antibodies that activate latent TGF-β1 by binding to GARP/latent TGF-β1 complexes, to provide means to stimulate Treg functions *in vivo*. If we succeed, we will attempt to obtain the crystallographic structure of GARP/latent TGF-β1 in complex with our activating antibody. We intend to identify the tri-dimensional changes in GARP/latent TGF-β1 complexes that lead to the release of active TGF-β1 and obtain information on the mode of action of the antibody. We tested 81 monoclonal antibodies directed against GARP, latent TGF-β1 or GARP/latent TGF-β1 complex *in vitro*. Only 7 were capable of activating TGF-β1 from GARP/latent TGF-β1 complexes. All these antibodies bind murine latent Tgf-β1 regardless the presence of Garp. After analysis of binding and activating properties of these anti-mouse latent Tgf-β1 antibodies, we started to confirm their activity *in vitro* on murine cell populations expressing Garp/latent Tgf-β1 complexes such as CD4+ T lymphocytes or platelets.

P.C4.03.12

TOLERance-induction with autologous tolerogenic dendritic cells treated with VITamin D3 and loaded with myelin peptides in Multiple Sclerosis (TOLERVIT-MS trial): The design of a multicenter, dose-escalation phase I clinical trial

M. Mansilla[1], *S. Presas-Rodríguez*[2], *J. Navarro-Barriuso*[1], *A. Teniente-Serra*[1], *B. Quirant-Sánchez*[1], *A. Lopez-Díaz de Cerio*[3], *S. Inogés*[3], *F. Prósper*[3], ***E. Martínez-Cáceres***[1], *C. Ramo-Tello*[2];
[1]*Germans Trias i Pujol Hospital and Research Institute. Campus Can Ruti, Badalona, Spain,* [2]*Germans Trias i Pujol Hospital, Badalona, Spain,* [3]*Cell Therapy Center, Clínica Universidad de Navarra, Pamplona, Spain.*

Tolerogenic dendritic cell (tolDC) therapy is a promising strategy for the attenuation of pathogenic T cells in autoimmune diseases such as multiple sclerosis (MS). Our group has developed an autologous antigen-specific cell therapy based on vitamin D3 (VitD3)-tolDC loaded with myelin peptides. **OBJECTIVE**: To describe the design of a multicenter, open-label, dose-escalation Phase I clinical trial to evaluate feasibility, safety, tolerability and preliminary efficacy of intranodal administration of VitD3-tolDC in active MS patients. **METHODS:** In vitro studies have demonstrated a potent immunoregulatory activity of vitD3-tolDC reducing lymphocyte proliferation and IFN-g production and increasing IL-10 levels, in co-culture experiments. Moreover, in vivo studies in the animal model of MS revealed a beneficial effect of VitD3-tolDC ameliorating the severity of the disease. Considering these pre-clinical results, a clinical trial was designed. **RESULTS**: Active MS patients will be included in a dose-escalation best-of-five design: Cohort 1 (5x10^6 VitD3-tolDC), Cohort 2 (10x10^6), Cohort 3 (15x10^6). A fourth Cohort of patients under IFN-beta treatment receiving the selected dose of VitD3-tolDC will be included. The trial protocol has been approved by the Spanish regulatory authorities (AEMPS) (https://clinicaltrials.gov/ct2/show/NCT02903537). Each cohort will received 6 administrations of tolDC (first 4 every 2 weeks and last 2 every 4 weeks). Clinical, MRI and immunological monitoring of 12 patients will be performed for 24 months. Each patient will be its own pre- and post-intervention control. **CONCLUSIONS:** Positive outcomes of this phase I clinical trial may lead to a phase II trial to investigate the efficacy of this therapy in MS patients

P.C4.03.13

TNFR2 blockade on Treg unleashes anti-tumor immune response after hematopoietic stem cell transplantation

A. Moatti[1,2], *A. Debesset*[1,2], *C. Pilon*[1,3,2], *S. Maury*[1,2,3], *J. Cohen*[1,2,3], *A. Thiolat*[1,2];
[1]*Inserm, U955, Equipe 21, Créteil, France,* [2]*Université Paris-Est, UMR_S955, UPEC, Créteil, France,* [3]*APHP, Hôpital H. Mondor-A. Chenevier, Centre d'Investigation Clinique Biothérapie, Créteil, France.*

Background. Allogeneic hematopoietic stem cell transplantation (alloSCT) was the first immunotherapy developed to treat hematological malignancies. Even if the graft-versus-tumor (GVT) effect of alloSCT relies on effector donor T cells (Teffs), regulatory T cells (Tregs) also present in the graft could minimize GVT. In mouse and human, Tregs highly express the TNFα type 2 receptor (TNFR2) and TNFR2+Tregs display a strong suppressive capacity. Importantly, by blocking the TNF/TNFR2 pathways, we recently revealed a complete TNF dependency for Treg in terms of suppressive capacity in vivo after alloSCT. Here, we hypothesize that the disruption of the TNF/TNFR2 pathway could be use to set off an efficient GVT effect after alloSCT.
Methodology. B6D2F1 irradiated mice were receiving B6 bone marrow cells plus sub-optimal numbers of donor T cells that were not capable to eliminate the murine mastocytoma cell line (P815) administered at time of alloSCT. 500µg of anti-TNFR2 blocking mAb were administered at days 0, 2 and 4. Mice were monitored for clinical and biological tumor emergence.
Results. In the blood of grafted mice receiving a sub-optimal T cell dose, P815 cells were detected and almost all mice died from tumor development. Despite TNFR2 expression on P815 cell surface, anti-TNFR2 treatment did not allow tumor elimination. In contrast, 64% of anti-TNFR2 treated mice were free of P815 attesting of a strong GVT effect.
Conclusion. Targeting TNFR2 can be a promising strategy to impact the Teff/Treg equilibrium in the favor of an improved alloreactivity and a better anti-tumor effect.

P.C4.03.14

Study of autoantibodies in limbic encephalitis and their relationship with paraneoplastic syndromes

I. Olivas Martinez, *L. Gonzalez Garcia, M. Montes Cano, E. Franco Macias, A. Palomino Garcia;*
Hospital Virgen del Rocio, Sevilla, Spain.

This work had as objective to do a retrospective study in patients with a suspected autoimmune encephalitis, analyzed during 2016-2017 period, looking for a possible association with paraneoplastic syndromes, commonly related to this kind of immune disorders. We studied serum and/or cerebrospinal fluid (CSF) from 113 patients, 58 men and 55 women with ages between 3 and 80, for a 2-year period. These samples were analyzed by indirect immunofluorescence to detect autoantibodies against surface neuronal antigens. First, a screening was done using cerebellar and hippocampus tissue, as well as cells transfected with glutamate receptor (NMDAR). Positive samples were subjected to a second study with a biochip containing cells transfected with six different surface neuronal antigens: NMDA-R, AMPAR1/2, CASPR2, DPPX, LGI1 Y GABARB1/2. Finally, clinical data of positive patients were contrasted searching a possible primary tumor that could be indicative of paraneoplastic syndrome. A total of nine patients (8%) showed antibodies for surface neuronal antigens. Six out of these 9 presented antibodies for NMDAR (67%), 1 for CASPR2 (11%), 1 for LGI1 (11%) and the other (11%) didn't show any associated specificity after a positive screening against hippocampus. Three patients (33%) suffered from a primary ovarian or breast cancer in the context of a paraneoplastic syndrome, all of them with anti-NMDAR antibodies (50% of patients with these antibodies). Detection of autoantibodies against surface neuronal antigens can lead to early identification and diagnosis of paraneoplastic syndromes, thus allowing the application of suitable treatments for primary tumors and neurological symptoms of limbic encephalitis.

P.C4.03.15

Functional interaction of Bcl-3 and IkBNS alters Treg cell development

C. Plaza Sirvent[1,2], *A. Matthies*[1,2], *M. Schuster*[1,2], *I. Schmitz*[1,2];
[1]*Otto-von-Guericke-Universität, Magdeburg, Germany,* [2]*Helmholtz Centre for Infection Research, Braunschweig, Germany.*

Regulatory T (Treg) cells are essential to maintain immune homeostasis. NF-κB proteins participate in the regulation of the Treg master transcription factor Foxp3. NF-κB proteins are regulated by nuclear proteins, called atypical IκB proteins, which modulate the transcriptional activity of NF-κB. We have previously demonstrated that IκBNS, an atypical IκB, drives the expression of Foxp3 via the association with the Foxp3 locus. Thymic Treg cells develop from $CD4^+$ $CD8^-$ thymocytes, which upregulate first GITR (Treg pre-precursor) and later CD25 (Treg precursor). Afterwards, Foxp3 is expressed and these cells are considered Treg cells. IκBNS-deficient mice show a blockage in the Treg precursor stage ($CD4^+$ $CD8^-$ $GITR^+$ $CD25^+$ $Foxp3^-$) of the thymic development, resulting in a 50 % reduction in the peripheral Treg population. Strikingly, these mice do not develop any autoimmune disorder. Other investigators have shown that overexpression of Bcl-3, another atypical IκB protein, affects Treg development and function causing spontaneous colitis in mice. In our current work, we demonstrate that the deficiency of the atypical proteins Bcl-3 and IκBζ do not have any impact on Treg development. Interestingly, despite the normal Treg development in Bcl-3-deficient mice, the combined absence of Bcl-3 and IκBNS causes Treg development blockage in an earlier stage compared to the single IκBNS-deficient mice. Moreover, these mice show signs of spontaneous immune activation in young mice suggesting functional impairment of Treg cells in double-deficient mice. Our data suggest that the functional interaction of the different atypical IκB proteins is important for Treg development and function.

P.C4.03.16

Investigating the cellular and molecular basis of bystander suppression in autoimmunity

***N. Richardson**, D. Wraith;*
Institute of Immunology and Immunotherapy, Birmingham, United Kingdom.

Induction of tolerance by peptide immunotherapy towards a single epitope of an antigen dampens the immune response not only of CD4+ T cells specific to tolerised antigen, but also of pathogenic T cells specific for other spatially-related auto-antigens. The tolerisation protocol extends tolerance from a single epitope to multiple disease-relevant epitopes by the mechanism of bystander suppression. Therefore, clinical application of peptide immunotherapy could have therapeutic potential for complex autoimmune diseases characterised by reactivity to multiple antigens.
Damage to the myelin sheath in multiple sclerosis is mediated by T cells targeting a number of myelin proteins. We have found that tolerised MBP-specific CD4+ T cells are able to exert bystander suppression to MOG-specific cells *in vivo*. When Tg4 mice are tolerised to MBP, then challenged with a MBP-MOG hybrid peptide, responses to both peptides are reduced. The coupling of the epitopes with a four amino acid linker domain mimics spatial proximity of disease-mediating antigens.
We are currently exploring the specific phenotype of the induced, regulatory CD4+ T cells, and the roles of TGF-β, IL-10, IL-4 and co-inhibitory receptors in the mechanism of bystander suppression in this model.

P.C4.03.17

Beneficial effect of probiotic *Lactobacillus kefiri* in lipopolysaccharide induced preterm birth in mice

***M. VENTIMIGLIA**;*
Centro de Estudios Farmacologicos y Botanicos-CONICET, Buenos Aires, Argentina.

Preterm birth (PTB) is a recurrent complication of pregnancy affecting 5-18% of all births worldwide and leading to serious consequences for the mother and the progeny. Although aetiology is not fully determinate, it is known that inflammation is a contributing factor to PTB. Probiotics were proved to be able to modulate immune responses promoting anti-inflammatory environments. Hence, they are being used in prevention and treatment of infectious diseases and immunopathologies. The aim of this work was to study the effects of *L.kefiri* on lipopolysaccharide (LPS) induced PTB in mice. C57BL76 females were administered every 48h by oral gavage during a week with probiotic (PB) or vehicle. Afterward, animals were mated with BALB/c males. Day of the vaginal plug detection was considered day 0.5 of pregnancy. Treatment continued every 48h during pregnancy, and females were challenged with LPS on gd 16.5. Births before gd 18.5 were considered PTB. Mice from control group given LPS exhibited 100% of PTB, with a majority delivering dead pups or live pups prematurely. Remarkably, treatment with probiotic completely protected pregnant mice to develop LPS-induced PTB with a majority delivering viable term pups. Additionally, while uterus from PB mice looked healthy, uterus from control mice exhibited haemorrhagic areas and implantations sites were barely recognized. Pups from PB mice were bigger at birth and also gained higher weight during lactation period compared to pups from control mice. Our results demonstrate that *L.kefiri* prevents LPS-induced PTB, opening new avenues to explore its therapeutic use for preventing PTB in humans.

P.C4.03.18

CD4+ Foxp3+ regulatory T cell-mediated immunomodulation by pharmacological inhibition of the acid sphingomyelinase in humans

***T. Wiese**[1], F. Dennstädt[1], C. Hollmann[1], S. Stonawski[2], C. Wurst[2], M. Buttmann[3,4], A. Menke[2], J. Schneider-Schaulies[1], N. Beyersdorf[1];*
[1]University of Würzburg, Institute for Virology and Immunobiology, Versbacher Str. 7, Würzburg, Germany, [2]University Hospital Würzburg, Department of Psychiatry, Psychosomatics and Psychotherapy, Margarete-Höppel-Platz 1, Würzburg, Germany, [3]Caritas Hospital Bad Mergentheim, Department of Neurology, Uhlandstraße 7, Würzburg, Germany, [4]University Hospital Würzburg, Department of Neurology, Josef-Schneider-Str. 11, Würzburg, Germany.

The acid sphingomyelinase (ASM) is a key modulator of cellular signaling pathways in which bioactive sphingolipids play crucial roles by catalyzing the cleavage of sphingomyelin to ceramide and phosphocholine. Recently, we have demonstrated that genetic deficiency for or pharmacological inhibition of the ASM increases the activity and frequency of mouse Foxp3$^+$ regulatory T cells (Treg) among CD4$^+$ T cells. Furthermore, pharmacological inhibition of the ASM had beneficial effects in different mouse models of autoimmune and inflammatory diseases. In the present study, we performed *in vitro* experiments with human T cells using two widely prescribed antidepressants with high (sertraline) or low (citalopram) capacity to inhibit ASM activity. Similar to our findings in mice, ASM inhibition in human PBMC increased the frequency of Treg among human CD4$^+$ T cells. To assess whether these effects on human Treg are transferable *in vivo,* we have been prospectively analyzing the composition of CD4$^+$ T cells in patients treated for major depression. Our preliminary data show that pharmacological inhibition of the ASM is superior to anti-depressants with little or no ASM-inhibitory activity in normalizing effector Treg frequencies among CD4$^+$ T cells in patients treated for depression. In summary, we find that inhibition of the ASM increases the frequency of (effector) Treg among CD4$^+$ T cells in mice and humans suggesting that ASM blockade might beneficially modulate autoimmune diseases and depression-promoting inflammatory reactions. This study was supported by a grant from the DFG (FOR2123 project P02).

P.C4.03.19

The effect of IL-2/anti-IL-2 complex treatment on antigen presenting cells

***M. Wiletel**[1], B. Mahr[1], N. Granofszky[1], M. Muckenhuber[1], T. Wekerle[1], J. Sprent[2], N. Pilat[1];*
[1]Department of Surgery, Medical University of Vienna, Vienna, Austria, [2]Immunology Division, Garvan Institute of Medical Research, Sydney, Australia, Sydney, Australia.

Introduction: The use of Interleukin-2 (IL-2) complexed with a specific antibody against IL-2 (IL-2cplx) has been shown to selectively increase regulatory T cells (Tregs) without significant proliferation of other IL-2 responsive immune cells. Here we focused on the effect of IL-2cplx treatment on antigen presenting cells (APCs) and changes in the expression of markers known to be relevant for immune response activation.
Methods: C57BL/6 mice received IL-2cplx (1µg/5µg), i.p., for 3 consecutive days and were sacrificed on day 5. We used flow-cytometric analysis to investigate the frequency of Tregs and APCs focusing on the expression of relevant markers on APCs for immune response activation, namely CD80, CD86, PDL-1 and MHC class II in samples taken from spleen.
Results: We demonstrate that IL-2cplx led to significant expansion of Tregs (17.88% vs 4.3% p=0.00006; vs naïve) and changes in the frequency of CD11c+ APCs (2.01% vs 0.94% p=0.005; vs naïve). Moreover we demonstrated significantly lower expression of CD80 and CD86 (16.4% vs 22.8% p=0.02 and 29.8% vs 34% p=0.09; vs naïve) and significantly higher expression of MHC class II on CD11c+DCs (82.9 % vs 75.35% p=0.009; vs naïve).
Conclusion: Treatment with IL-2cplx and subsequent expansion of Tregs leads to reduced expression of CD80+ and CD86+ but increased MHC class II expression on APCs which could cause impaired effector T cell function. This may highlight possible ligand-receptor interactions and help to understand important cellular key mechanism mediated by APCs and T cells.

P.C4.03.20

Treatment of collagen-induced arthritis (CIA) in mice model with disease-inducible indoleamine-2,3-dioxygenase (IDO) gene

***I. Yilmaz**[1], G. Guvenc[2], M. Karacay[1], A. O. Barazi[3], F. Ersoy[4], C. Akkoc[5], A. Akkoc[6], A. Yilmaztepe Oral[7], M. Yalcın[8], H. B. Oral[9];*
[1]Department of Medical Immunology, Institute of Health Sciences, Uludag University, Bursa, Turkey, [2]Department of Veterinary Physiology, Institute of Health Sciences, Uludag University, Bursa, Turkey, [3]Department of Immunology, Institute of Health Sciences, Gazi University, Ankara, Turkey, [4]Department of Molecular Biology & Genetics, Faculty of Arts & Sciences, Uludag University, Bursa, Turkey, [5]Department of Histology and Embryology, Faculty of Veterinary, Uludag University, Bursa, Turkey, [6]Department of Pathology, Faculty of Veterinary, Uludag University, Bursa, Turkey, [7]Department of Medical Biochemistry, Faculty of Medicine, Uludag University, Bursa, Turkey, [8]Department of Physiology, Faculty of Veterinary, Uludag University, Bursa, Turkey, [9]Department of Immunology, Faculty of Medicine, Uludag University, Bursa, Turkey.

In RA patients, tryptophan catabolism has an impact on progression and indoleamine-2,3-dioxygenase (IDO) has a crucial role in the induction of immune tolerance. In this study, the treatment responses of disease-inducible IDO gene over expression in CIA mice were investigated. Human IDO cDNA mammalian expression plasmid (pCMV/hIDO) CMV promoter (constantly active) was replaced with pELAM-1 promoter (only active in the presence of inflammatory cytokines) which was taken out from pELAM-1pro/CAT plasmid. HeLa cells were transfected with pELAM-1pro/hIDO and induced with IL-1β for 6 or 24 hours. hIDO levels were determined from the cell lysate. The combination of therapeutic plasmid and controls with liposomes intraarticularly delivered after arthritis developed in mice and followed up for 4 weeks. The therapeutic effects evaluated by considering ankle circumference, clinical and histopathological scoring of mice. Moreover, the difference between CD4+ T cell and CD68+ synovial macrophages amounts, and IDO expression in joints was examined. It was observed that hIDO was significantly increased following 6 and 24 hours stimulation with IL-1β in HeLa cells transfected with pELAM-1pro/hIDO. Ankle circumference, clinical and histological scores decreased in CIA mice which are treated with pELAM-1pro/hIDO. The ratios of CD4$^+$ T cells and CD68$^+$ synovial macrophages decreased and IDO levels increased following pELAM-1pro/hIDO treatment. Thus, conditional targeting IDO can be a new approach for the treatment of RA. This study is supported by The Scientific and Technical Research Council of Turkey (TUBITAK-COST Project No: 113S375) under Cost Action BM1305. COST is supported by the EU Framework Programme Horizon 2020.

P.C5.01 Allergy, asthma and therapy - Part 1

P.C5.01.01
Loading of the lipocalin BLG with iron-quercetin complexes prevents the onset of allergy in a BALB/c mouse model

S. M. Afify[1], *I. Pali-Schöll*[2], *G. Hofstetter*[2], *A. Vidovic*[2], *L. Pacios*[3], *F. Roth-Walter*[2], *E. Jensen-Jarolim*[4];
[1]*The Interuniversity Messerli Research Institute of the University of Veterinary Medicine Vienna, Medical University Vienna and University Vienna, Vienna, Austria and Laboratory Medicine and Immunology Department, Faculty of Medicine, Menoufia University, Menoufia, Egypt,* [2]*The Interuniversity Messerli Research Institute of the University of Veterinary Medicine Vienna, Medical University Vienna and University Vienna, Vienna, Austria,* [3]*Biotechnology-Vegetal Biology Department, ETSIAAB and Center for Plant Biotechnology and Genomics (CBGP, UPM-INIA), Technical University of Madrid, Madrid, Spain,* [4]*The Interuniversity Messerli Research Institute of the University of Veterinary Medicine Vienna, Medical University Vienna and University Vienna and Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Vienna, Austria.*

Background: Prevention of milk allergy is an urgent problem that has attracted the attention of food scientists. In previous studies we proved that the unloaded apo-form of the lipocalin beta-lactoglobulin (BLG) from milk promoted Th2 cells and inflammation, whereas the holo-form acted immunosuppressive. In this study, we tested in BALB/c mice whether nasal application of holo-BLG can prevent allergy to BLG.
Methods: BALB/c mice were sensitized twice intraperitoneally with BLG adjuvanted with aluminum hydroxide after being nasally treated 3 times in biweekly intervals with the unloaded apo-form of BLG, or holo-BLG loaded with quercetin-iron complexes, or water as sham-treatment. Then mice were intraperitoneally challenged with apo-BLG. Subsequently, body temperature drop was recorded as a sign of a systemic allergic reaction. Specific antibodies in serum as well as cytokines of BLG-stimulated splenocytes were analyzed by ELISA. MHC Class II I-Ad+ and CD86+ expression on CD11c+ dendritic cells from spleens were analyzed by flow cytometry.
Results: Intranasal prophylactic treatment with holo-BLG prevented allergic sensitization to BLG. Mice pretreated with water or apo-BLG had significantly elevated BLG-specific antibodies (IgG1, IgG2a, IgA and IgE), cytokine levels (IL5, IL13 and IL10) and significantly upregulated MHC Class II I-Ad and CD86+ on CD11c+ dendritic cells in the spleens, compared to holo-BLG treated group. Pretreatment with holo-, but not apo-BLG prevented body temperature drop upon allergen-challenge.
Conclusion: Prophylactic treatment with holo-BLG provided specific protection against sensitization to BLG and prevented the onset of allergy.
Grants: FWF, grant SFB F4606-B28, Austria and a grant from Egypt to SMA.

P.C5.01.02
Analysis of IRF4-binding characteristics using a streptavidin-biotin-dependent "bio-ChIP"

S. Dietzen*, T. Bopp;*
University Medical Center of the Johannes Gutenberg-University, Institute for Immunology, Mainz, Germany.

Mast cells as well as IL-9 play a crucial role in the pathogenesis of asthma and recent findings demonstrated that the transcription factor IRF4 regulates the *Il9* gene expression in mast cells. We could demonstrate that IRF4 transactivates the *Il9* promoter and IRF4 deficiency leads to an impaired IL-9 production. The aim of the project is to analyze IRF4-binding characteristics, thus identify the concrete binding sites of IRF4 in the genome and describe physical interaction partners of the transcription factor. To this end, we established a transgenic mouse which allows constitutive *in vivo* biotinlyation of IRF4. We used a mouse expressing the BirA ligase (an enzyme which catalyzes the biotinylation of proteins that are tagged with a specific recognition sequence) and crossed it to a mouse expressing IRF4 fused to the BirA ligase recognition sequence as a transgene. Breeding of this mouse strain allows the generation of a mouse model where IRF4 will be biotinylated *in vivo*. To identify IRF4 binding sites within the *Il9* promoter and also the whole genome, mast cells from the IRF4-transgenic mice shall be used to establish a so called "bio-ChIP" that allows for precipitation of IRF4-bound DNA elements by using streptavidin-coated magnetic beads instead of protein-specific antibodies. Upon NGS-based sequencing, this method can be used for genome-wide analyses of IRF4-bound genes. Moreover, data achieved by purification of IRF4 in combination with co-binding proteins and following analysis via western blot and mass spectrometry will help to enlighten further details of the regulatory network of IRF4.

P.C5.01.03
Helminths protect from basophil- and eosinophil-mediated chronic allergic skin inflammation.

J. U. Eberle*, D. Voehringer;*
Department of Infection Biology, University Hospital Erlangen and Friedrich-Alexander University Erlangen-Nuremberg (FAU), Erlangen, Germany.

Introduction: Basophils are required for IgE-mediated chronic allergic skin inflammation (IgE-CAI) in the mouse and promote the accumulation of eosinophils in tissues. However it is not clear how eosinophils are recruited and if helminth-elicited IgE is protective in this setting.
Material and Methods: Here, we used an antibody-induced model of IgE-CAI in mice. 33µg IgE antibody against TNP was injected intravenously followed by intradermal injection of 10µg TNP-OVA in the ear the next day. We measured changes in ear thickness and recruitment of effector cells into the ear parenchyma and compared helminth infected vs. naïve and several transgenic mouse strains.
Results: The ear swelling was absent in basophil- and eosinophil-deficient mice as well as in mice lacking the high affinity receptor for IgE on basophils. Swelling was decreased in mice infected with *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus* compared to wildtype mice. Further IL-4/IL-13-deficient mice, as well as Stat6-deficient mice showed slightly decreased ear swelling. Infiltration of basophils, eosinophils, and neutrophils was also impaired.
Conclusion: From our studies we can conclude that in IgE-CAI basophils are activated via the IgE receptor and promote the recruitment of eosinophils which are required for pathology. Saturation of basophils with Helminth-elicited IgE appears to protect from IgE-CAI.

P.C5.01.04
A single high-dose feeding with the major carp allergen parvalbumin induces immunological and clinical tolerance in a mouse model of fish allergy

R. Freidl[1], *A. Gstoettner*[1], *U. Baranyi*[2], *I. Swoboda*[1], *G. Stavroulakis*[3], *N. Papadopoulos*[3,4], *F. Stolz*[5], *M. Focke-Tejkl*[1], *T. Wekerle*[2], *R. van Ree*[6], *R. Valenta*[1], *B. Linhart*[1];
[1]*The Division of Immunopathology, Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria,* [2]*The Section of Transplantation Immunology, Department of Surgery, Medical University of Vienna, Vienna, Austria,* [3]*Allergy Research Center, 2nd Pediatric Clinic, University of Athens, Athens, Greece,* [4]*Division of Infection, Immunity & Respiratory Medicine, University of Manchester, Manchester, United Kingdom,* [5]*Biomay AG, Vienna, Austria,* [6]*The Departments of Experimental Immunology and of Otorhinolaryngology, Academic Medical Center, Amsterdam, Netherlands.*

Introduction: IgE-mediated food allergy to fish is a persistent, potentially life-threatening hypersensitivity disease. IgE-sensitization to food allergens is is detectable already at three months of age. Thus a prophylactic strategy for prevention of IgE-sensitization to food allergens in infants is the ultimate goal. This study sought to investigate the effect of feeding of natural carp parvalbumin (nCyp c 1) on the antibody responses and allergic symptoms in a fish allergy mouse model. Materials and methods: BALB/c mice (n=8) were fed with 10mg nCyp c 1 or PBS (day 1). Following feeding mice were immunized twice subcutaneously with 20µg nCyp c 1 adsorbed to aluminum-hydroxide in a 2-week interval. Control groups were sensitized to Cyp c 1 without gavage or received gavage only. Upon intragastric allergen challenge with nCyp c 1 allergic symptoms and body temperature were recorded.
Cyp c 1-specific antibody responses were analyzed in ELISA and rat basophil leukemia assay. Results: Measurement of Cyp c 1-specific antibodies demonstrated that IgE-sensitization was prevented by prophylactic feeding with nCyp c 1. Further, mice fed with nCyp c 1 before sensitizations were protected from symptom-development upon allergen challenge. Conclusions: A high-dose feeding with carp parvalbumin induced tolerance in our mouse model. The induction of clinical tolerance to allergens was investigated in numerous oral immunotherapy trials. However, the application of natural allergens causes severe symptoms in patients and clinical tolerance is often not persistent. We suggest prophylactic tolerance induction as alternative strategy. Support: FAST-project 201871 and Austrian Science Fund-projects P23350-B11, F4605.

P.C5.01.05
Comprehensive tracking of mediator reprogramming in type 2 immune settings reveals macrophage eicosanoid plasticity during allergen exposure

A. Friedl[1], *F. Henkel*[1], *D. Thomas*[2], *T. Bouchery*[3,4], *P. Haimerl*[1], *C. B. Schmidt-Weber*[1], *J. Adamski*[5,6], *N. L. Harris*[3,4], *M. Haid*[5], *J. Esser-von Bieren*[1];
[1]*Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Center Munich, Munich, Germany,* [2]*Pharmazentrum Frankfurt/ZAFES, Institute of Clinical Pharmacology, Goethe University Frankfurt, Frankfurt, Germany,* [3]*Laboratory of Intestinal Immunology, Global Health Institute, School of Life Sciences, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland,* [4]*Department of Immunology and Pathology, Faculty of Medicine, Nursing and Health Science, Monash University, Melbourne, Australia,* [5]*Institute of Experimental Genetics, Genome Analysis Center, Helmholtz Center Munich, Neuherberg, Germany,* [6]*Chair of Experimental Genetics, Life and Food Science Center Weihenstephan, Technical University of Munich, Freising-Weihenstephan, Germany.*

Background: Eicosanoid lipid mediators play key roles in allergy and asthma. Macrophages represent major cellular sources of these mediators, but their complex and dynamic eicosanoid output during type 2 immune responses is not understood. Objective: We aimed to comprehensively track lipid mediator production in type 2 immune responses. Methods: We established an LC-MS/MS workflow for the quantification of 52 oxylipins to track lipid mediator reprogramming in human monocyte derived macrophages (MDM) during exposure to house dust mite (HDM) or in nematode infection *in vivo*. Eicosanoid enzymes were studied by qPCR and westernblot and cytokine production was assessed by multiplex assays. Results: Differentiation of macrophages with GM-CSF and TGFβ1 resulted in a phenotype ("aMDM") with characteristic features of airway macrophages such as high expression of 5-lipoxygenase (5-LOX), which resisted IL-4-mediated transcriptional repression. Exposure of aMDM to HDM resulted in the suppression of 5-LOX expression and product formation.

In contrast, HDM triggered increased prostanoid production with thromboxane and prostaglandins D_2 and E_2 as major metabolites. HDM also induced pro-inflammatory cytokines and chemokines, resulting in an overall M1-like mediator profile.
Finally, distinct changes in lipid mediator profiles occurred during the type 2 immune response to nematodes *in vivo*. Conclusion: Our findings show that type 2 immune responses are characterized by fundamental reprogramming of the lipid mediator metabolism with macrophages representing particularly plastic responder cells. Targeting mediator reprogramming in airway macrophages may represent an approach to regulate pathogenic lipid mediator profiles in allergy or asthma. Funding: German Research Foundation (DFG), Else Kröner-Fresenius-Stiftung

P.C5.01.06
Hapten-induced contact hypersensitivity is upregulated in interleukin-19 knockout mice

***Y. Fujimoto**, Y. Azuma;*
Laboratory of Veterinary Pharmacology, Division of Veterinary Science, Graduate School of Life and Environmental Science, Osaka Prefecture University, Izumisano, Osaka, Japan.

Interleukin-19 is a member of the IL-10 family of interleukins and is an immuno-modulatory cytokine produced by the main macrophages. The gastrointestinal tissues of IL-19 knockout mice show exacerbated experimental colitis mediated by the innate immune system and T cells. There is an increasing focus on the interaction and relationship of IL-19 with the function of T cells. Contact hypersensitivity (CHS) is T cell-mediated cutaneous inflammation. Therefore, we asked whether IL-19 causes CHS. We investigated the immunological role of IL-19 in CHS induced by 1-fluoro-2,4-dinitrofluorobenzene as a hapten. IL-19 was highly expressed in skin exposed to the hapten, and ear swelling was increased in IL-19 knockout mice. The exacerbation of the CHS response in IL-19 knockout mice correlated with increased levels of IL-17 and IL-6, but no alterations were noted in the production of IFN-gamma and IL-4 in the T cells of the lymph nodes. In addition to the effect on T cell response, IL-19 knockout mice increased production of inflammatory cytokines. These results show that IL-19 suppressed hapten-dependent skin inflammation in the elicitation phase of CHS.

P.C5.01.07
The impact of glycation on dendritic cell responses to proteins

***S. L. Harris**[1], F. Manodoro[2], A. G. Rust[2], R. Chauhan [2], N. Matthews[2], A. E. Moghaddam[1], Q. J. Sattentau[1];*
[1]The Sir William Dunn School of Pathology, Oxford, United Kingdom, [2]The Institute of Cancer Research, London, United Kingdom.

Introduction: Advanced Glycation End products (AGEs) are a group of protein modifications formed by the non-enzymatic reaction between a reducing sugar and a free amine group. AGEs are formed endogenously and in food, for example during dry roasting of peanuts. AGE-modified proteins, including dry-roasted peanuts, have an enhanced immunogenicity compared to unmodified proteins and induce a Th2 bias. However, dry-roasted peanut and other glycated proteins do not induce conventional activation of dendritic cells (DCs). We hypothesised AGE-modified proteins may instead induce a non-canonical DC activation.
Materials and Methods: murine bone marrow derived DCs were pulsed with either dry-roasted or raw peanut proteins, AGE-modified egg allergens (hen egg lysozyme (HEL) or ovalbumin) or low dose lipopolysaccharide. Supernatants were collected to assess cytokine/chemokine secretion. DCs pulsed with AGE-modified HEL were also used for RNAseq.
Results: The transcriptomic and proteomic analyses demonstrated that modified proteins induced changes which were distinct from those elicited by the classical inflammatory stimulus lipopolysaccharide. There was a significant change in the expression of a small group of gene in response to HEL-Glucose. Pathway analysis using these results implicated a number of pathways, including those involved in the cell cycle and metabolism. Changes in secretion depended on both the modification type and the base protein.
Conclusions: Our results provide initial evidence for an alternative mode of DC activation by AGE-modified proteins. The variable secretory profiles induced by modified proteins indicate that multiple receptors are involved in these responses. Future studies will examine these potential receptors and their signalling pathways.

P.C5.01.08
Complement drives IgE-mediated experimental food allergy through the C5a/C5aR1 axis

***A. Kordowski**[1], A. T. Reinicke[1], D. Wu[2], J. Lee[2], Y. Wang[2], S. P. Hogan[2], J. Köhl[1];*
[1]University of Lübeck, Lübeck, Germany, [2]Cincinnati Childrens's Hospital and University of Cincinnati, College of Medicine, Cincinnati, United States.

Food-induced anaphylaxis is a serious allergic reaction caused by antigen cross-linking of IgE-loaded Fcε-receptors on mast cells (MCs), leading to the release of pro-inflammatory mediators and disease manifestation.The exact mechanisms breaking oral tolerance and the effector pathways driving food allergy remain elusive. As complement activation occurs in food-induced anaphylaxis, we aimed to assess the role of C5a in disease pathogenesis. BALB/c wildtype (wt) and *C5ar1*$^{-/-}$mice were subjected to oral antigen-induced food allergy model. Readouts included diarrhea development, changes in rectal temperature, hematocrit, antigen-specific serum IgE, MCPT-1 and intestinal MC numbers as well as FcεR1-mediated MC functions including C5a receptor 1 (C5aR1) regulation. Further, histamine-mediated hypothermia and regulation of endothelial tight junctions was determined. Repeated oral OVA challenge resulted in diarrhea, hypothermia, increased hematocrit, high OVA-specific serum IgE and MCPT-1 levelsin wt mice. In contrast, male *C5ar1*$^{-/-}$mice were completely whereas female *C5ar1*$^{-/-}$were partially protected from anaphylaxis development. The lower incidence of diarrhea in *C5ar1*$^{-/-}$mice was associated with decreased OVA-specific serum IgE in male and mast cell activation (MCPT-1 levels) in both sexes. Mechanistically, IgE-mediated degranulation and IL-6 production from *C5ar1*$^{-/-}$BMMCs of both sexes were significantly reduced. Importantly, FcεR1 cross-linking strongly upregulated C5aR1 MC expression *in vitro*and *in vivo*. Finally, histamine treatment resulted in a milder temperature drop in *C5ar1*$^{-/-}$males than in wt mice. Our findings identify C5aR1 activation as an important driver of IgE-mediated food allergy. C5aR1 targeting may prove useful to suppress the inflammatory response in food-induced anaphylaxis.

P.C5.01.09
Modulation of C5a receptor 2 expression in experimental allergic asthma

*I. Osman[1], K. M. Quell[1], A. V. Wiese[1], T. Vollbrandt[2], P. König[3], J. Köhl[1], **Y. Laumonnier**[1];*
[1]ISEF, Lübeck, Germany, [2]CANA Core, Lübeck, Germany, [3]Institute for Anatomy, Lübeck, Germany.

C5a drives airway constriction and inflammation during the effector phase of allergic asthma, mainly through the activation of C5a receptor 1 (C5aR1). In addition to C5aR1, C5aR2 (C5L2) regulates the allergic asthma phenotype. However its expression pattern on myeloid and lymphoid cells during the allergic effector phase is unknown. Recently, we generated and characterized a novel floxed tandem-dye Tomato fluorescent protein (tdTomato)-C5aR2 knock-in mouse, showing that C5aR2 expression is restricted to myeloid cells in the airways, the lungs and lymphoid organs. Using this reporter strain, we monitored C5aR2 expression during the effector phase of house dust mite-driven allergic asthma. C5aR2 reporter and wildtype mice developed an allergic phenotype with comparable airway resistance, mucus production, eosinophilic/neutrophilic airway inflammation and Th2/Th17 cytokine production. No major changes in C5aR2 expression occurred in myeloid cells during the allergic effector phase, in particular in airway eosinophils and a subset of neutrophils. However, we observed significant down regulation of C5aR2 on pulmonary macrophages. Lymphoid cells remained tdTomato-C5aR2 negative upon allergic inflammation except a small subset of NK cells, suggesting a functional role of C5aR2 in NK cells. In line with this observation, the NK cell marker NKp46 was strongly upregulated in C5aR2$^{-/-}$ NK cells. In summary, we show that C5aR2 expression is not altered in the allergic effector phase on most myeloid cells. In contrast, C5aR2 is downregulated in pulmonary macrophages. Further, it controls NKp46 expression in a subset of NK cells suggesting a novel function of C5aR2 in such cells during allergic inflammation.
Funding DFG-IRTG1911/A1

P.C5.01.10
A critical role of IL-18 in the maturation of PDL1 expressing pathogenic eosinophils

***A. Mishra**, S. Upparahalli Venkateshaiah, A. Mishra;*
Tulane University School of Medicine, New Orleans, LA, United States.

Eosinophils are multifunctional leukocytes with diverse functions in health and disease. We for the first time demonstrate that IL-18 has a critical role in the development and maturation of eosinophils. Herein, we provide evidence that IL-18 differentiates eosinophils, even in the absence of endogenous *IL-5,* both *ex vivo* and under physiological conditions (*in vivo*). IL-18 and IL-5 differentiated *ex vivo* eosinophils have differences in size, shape, granularity and differentially regulated *CD274 (PDL1)* transcript expression. Most importantly, we provide evidence that IL-18 is critical for transforming homeostatic eosinophils into mature *PDL1* expressing eosinophils. Notably, eosinophils with and without PDL1 expression are present in healthy mouse and human blood. Moreover, the proportion of eosinophils that express PDL1 is markedly increased in allergic mice and humans. Additionally, we report that all eosinophils in the lungs of asthmatic mice and the nasal lavage of asthmatic patients harbor PDL1 expressing pathogenic eosinophils. Analysis of mouse and human eosinophil data in healthy and disease state indicates that *IL-18* and *IL-5* synergistically promote differentiation, maturation and proliferation of *PDL1* expressing pathogenic eosinophils in allergic disease states. Enhanced IL-18 expression is reported in almost all allergic diseases and our data suggest that it has a critical role in eosinophil differentiation and the maturation of PDL1 expressing pathogenic eosinophils. Collectively, we first time identified the role of IL-18 in transforming naive eosinophils to pathogenic *PDL1* expressing eosinophils and this finding may have broad implications regarding non-invasive diagnostic and therapeutic strategies for eosinophil-associated diseases.

P.C5.01.11

A polymorphism rs928413 associated with asthma development and regulation of *IL33* gene expression

A. Muratova[1,2]*, N. Mitkin*[1]*, D. Kuprash*[1,2]*;*
[1]Engelhardt Institute of Molecular Biology Russian Academy of Sciences, Moscow, Russian Federation, [2]Biological Faculty, Lomonosov Moscow State University, Moscow, Russian Federation.

Cytokine IL-33 is secreted by epithelial and endothelial cells during necrosis and stimulates humoral immune response. Elevated IL33 expression in pulmonary epithelium of asthmatic patients correlates with exacerbation of allergen-induced inflammation and disease progression. We hypothesized that individual variations in the *IL33* gene expression may be explained by polymorphisms of non-coding regulatory regions, in particular by SNP rs928413 located in an promoter region in the *IL33* locus and associated with development of asthma.
Activities of the *IL33* promoter variants containing different rs928413 alleles were assessed upon transfection of the corresponding luciferase reporter constructs into NCIH pulmonary epithelial cell line followed by TNFα stimulation. CREB1 binding to DNA was estimated using pull-down assay.
We observed that rs928413 risk allele creates a functional binding site for CREB1, a transcription factor that stimulates expression of a number of inflammatory mediators. *IL33* promoter containing active CREB1-binding site showed significantly elevated activity in stimulated human lung carcinoma cells.
Our data suggest that differential binding of the rs928413 alleles to may underlie the emergence of asthmatic phenotype in response to increased *IL33* gene expression and of systemic inflammation.
This study is supported by grant 14-14-01140 from Russian Science Foundation.

P.C5.01.12

Sensitization state to Hymenoptera venom of an asymptomatic healthy beekeeper cohort

A. Navas[1]*, B. Ruíz*[2,1]*, M. Fisichella*[1]*, P. Serrano*[2,1]*, C. Moreno*[2,1]*, A. Jurado*[2,1]*;*
[1]Maimonides Biomedical Research Institute of Cordoba (IMIBIC)/ Reina Sofia University Hospital/ University of Cordoba, Cordoba, Spain, [2]Department of Allergy and Immunology, Reina Sofia University Hospital, Cordoba, Spain.

Allergy to Hymenoptera venom is a potentially mortal disease, being the specific immunotherapy the unique healing treatment. Beekeeping sector is the most vulnerable group to this health problem. There are not useful biomarkers to predict the response to stings or immunotherapy. Basophil activation test (BAT) has been correlated with medium and long-term immunotherapy effectiveness. The aim of this study was to evaluate the allergy immune state of a beekeeper cohort highly exposed to Hymenoptera venom but bee-sting tolerant, regarding a control healthy population using BAT.
We selected a cohort of 17 healthy beekeepers and 17 non-allergic control individuals. BAT was performed using Basotest (BD Biosciences). Total-blood samples were incubated with 0,1 and 1 µg/mL of *Apis mellifera* venom (Pharmalgen). Activated basophils were identified using CD123-PE, DR-PerCP and CD63-FITC monoclonal antibodies.
Interestingly, among 17 recruited healthy beekeepers, BAT was positive in 9 (52.9%) of them, while none of the healthy individuals exhibited a positive result in BAT ($p<0.001$). The average of $CD63^+$ cells degranulated against 1 µg/mL of venom was significantly different between beekeepers and healthy individuals (15.62% vs. 1.06%; $p=0.037$). No significant differences were found between groups when cells were stimulated with 0.1 µg/mL of venom ($p=0.407$).
Despite the fact that all recruited beekeepers were non-responders to Hymenoptera venom stings, more than the half were *in vitro* reactive by BAT. Therefore, the *in vitro* degranulation of basophils was not well associated with the immunological tolerance status observed in this cohort of individuals.

P.C5.01.13

Patients sensitized to the Ole e 7 lipid transfer protein from olive pollen, exhibit positive reactivity against Pru p 3 by basophil activation test

C. Oeo-Santos[1]*, A. Navas*[2]*, B. Ruíz*[2]*, M. Fisichella*[2]*, R. Barderas*[3]*, M. Villalba*[1]*, A. Díaz-Perales*[4]*, C. Moreno*[2]*, A. Jurado*[5]*;*
[1]Complutense University of Madrid, Madrid, Spain, [2]Maimonides Biomedical Research Institute of Córdoba (IMIBIC)/ Reina Sofía University Hospital/ University of Córdoba, Córdoba, Spain, [3]CROSADIS, Carlos III Health Institute, Madrid, Spain, [4]Center of Biotechnology and Genomics of Plants, Polytechnic University, Madrid, Spain, [5]Department of Allergy and Immunology, Reina Sofia University Hospital, Madrid, Spain.

Ole e 7 lipid transfer protein (LTP) from olive pollen is responsible for severe symptoms in the south of Spain. Many Ole e 7 sensitized patients exhibit co-sensitization to the peach LTP Pru p 3, despite there is no evidence of cross-reactivity which explains this co-sensitization to date. Herein, we present the preliminary results from basophil activation test (BAT) to Pru p 3 of sensitized patients to Ole e 7. IgE specific serum level to Ole e 7 and Pru p 3 were measured by ImmunoCAP 250 and ELISA. BAT was performed using Basotest (BD Biosciences). Total-blood samples were separately incubated with 10 µg/mL of Ole e 7 and Pru p 3 purified recombinant allergens. Activated basophiles were identified using CD123-PE, DR-PerCP and CD63-FITC monoclonal antibodies. None of the patients had been treated with immunotherapy for the last 3-years. The all four patients, who only had serum specific IgE against Ole e 7 (40.4±19.8 kU/L), exhibited basophil degranulation using Pru p 3 as stimulus. The average of $CD63^+$ cells after stimulating with Ole e 7 and Pru p 3 were 45% and 32.2%, respectively. Despite the small size of the study-cohort, we observed a double *in vitro* basophil degranulation using Ole e 7 and Pru p 3 as stimulus. However, no specific IgE sensitization to Ole e 7 was detected either by ELISA or ImmunoCAP. Further molecular studies should be performed to support the cross-reactivity observed in the present work.

P.C5.01.14

Naïve human T cells selectively differentiate into Th1 and Th17 cells after costimulation by human keratinocytes

C. Orlik[1]*, D. Deibel*[1]*, E. Balta*[1]*, B. Niesler*[2]*, Y. Samstag*[1]*;*
[1]Institute of Immunology, Heidelberg, Germany, [2]Institute of Human Genetic, Heidelberg, Germany.

Allergic contact dermatitis (ACD) corresponds to a delayed-type hypersensitivity response induced by repeated skin contact with low molecular weight chemicals (haptens). It shows common histological characteristics, e.g. cytopathic effects on keratinocytes and infiltration of T cells, monocytes as well as dendritic cells into the epidermis and dermis. In the pathophysiology of ACD, keratinocytes are critically involved by functioning as the primary barrier of the skin as well as by their ability to secrete pro-inflammatory mediators. However, the potential impact of keratinocytes on skin-infiltrating and skin-homing T cells, remained mainly elusive. Here we investigated the direct interaction of primary human keratinocytes with primary human T cells under ACD-related conditions by using an *in vitro* coculture system. We demonstrated that under pro-inflammatory conditions, keratinocytes may act as non-professional antigen presenting cells (APC) causing T cell activation. A more detailed analysis revealed that even naïve T cells can be activated through keratinocytes. By disrupting interactions between stimulatory coreceptors and their ligands in the *in vitro* coculture, the necessity of CD54 and CD58 for keratinocyte-induced T cell activation was demonstrated. Subsequent gene expression analysis showed a selective upregulation of IFNγ-regulated genes in these keratinocyte-activated naïve T cells. Interestingly, naïve T cells co-stimulated by keratinocytes selectively differentiated into Th1 and Th17. Furthermore, keratinocyte-induced T cell activation initiates the specific upregulation of skin-homing factors on naïve T cells. Thus, local interference with T cell-keratinocyte interactions may open up novel strategies for the treatment of skin-related diseases.

P.C5.01.15

Treatment with toxoplasma gondii tachyzoite lysate reduces allergic mono- and polysensitization and airway inflammation in mice

M. J. Orola[1]*, E. Korb*[1]*, M. Drinić*[1]*, T. Svoboda*[2]*, M. Ehling-Schulz*[2]*, I. Schabussova*[1]*, U. Wiedermann*[1]*;*
[1]Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Vienna, Austria, [2]Institute of Microbiology, Department of Pathobiology, University of Veterinary Medicine, Vienna, Austria.

Toxoplasma gondii is an obligatory intracellular protozoan parasite which can infect a range of hosts via ingestion of oocysts. Studies have shown that *T. gondii* infection can influence allergic sensitization and even suppress allergic airway inflammation in mouse models. Our previous work examined the properties and the immunological impact of the tachyzoites lysate antigen (TLA) which is derived from the differentiated state of the parasite.
The aim of this study was to further investigate the effects of TLA immunization through mucosal application upon OVA sensitization and challenge as well as polysensitization and challenge with the recombinant proteins Bet v 1, Phl p 1 and Phl p 5 in BALB/c mice.
In both settings, TLA ameliorated Th2-mediated airway inflammation, as indicated by the general decrease of IL-4 and IL-5 along with a reduction of IL-13 in the lungs. Similarly, eosinophilia was diminished, while expansion of macrophages and neutrophils were observed in the bronchoalveolar lavage. Moreover, FACS analysis from the polysensitization model revealed a significant increase of IL-17 producing $CD4^+$ T-cells in the lungs. TLA not only influences the immune system directly but also appears to alter the gut microbiota in the context of OVA sensitization, as indicated by Fourier transform-infrared spectroscopy. TLA application upon allergen challenge improved airway inflammation, further highlighting the potential benefits in treatment of allergy.
Supported by the FWF

P.C5.01.16

The mechanism of reactive oxygen species-induced apoptosis in mast cells in response to lysosomotropic agents

A. Paivandy[1]*, F. R. Melo*[1]*, G. Pejler*[1,2]*;*
[1]*Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden,* [2]*Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden.*

Mast cells are infamous for their detrimental roles in allergic reactions and inflammatory lung diseases, such as asthma. Therefore, efficient strategies to limit their adverse effects in such pathological settings are needed. We have previously reported that lysosomotropic agents, such as mefloquine, induce mast cell apoptosis through granule permeabiliation and enhanced production of reactive oxygen species (ROS). Here we sought to explore the underlying mechanism of ROS-induced apoptosis in mast cells upon mefloquine treatment. Interestingly, we found that the ROS production in response to mefloquine is diminished in mast cells deficient in serglycin proteoglycan, suggesting that mast cell granules (also known as secretory lysosomes) may play a role in the ROS production. Given that previous reports highlighted a role for lysosomal metal ions in ROS production, we next pre-incubated mast cells with chelators of iron (deferoxamine) and/or copper (tetrathiomolybdate), followed by mefloquine treatment and assessment of ROS level. We observed that deferoxamine- or tetrathiomolybdate-treated mast cells generated reduced level of ROS after mefloquine treatment, indicating that iron and copper ions are key component in mefloquine-induced ROS production in mast cells. Another potential source of ROS could be through the action of NADPH oxidase. However, our preliminary data show that inhibition of NADPH oxidase had little inhibitory effect on the ROS production in response to mefloquine, arguing against this possibility. Taken together, our preliminary data suggest that enhanced ROS production upon mefloquine treatment is likely the result of the Fenton reaction to a large extent.

P.C5.01.17

A fragment of extracellular matrix collagen drives epithelial remodelling and airway hyper-responsiveness in allergic airways disease

D. F. Patel[1]*, T. Peiro*[1]*, A. Shoemark*[1]*, S. Akthar*[1]*, S. A. Walker*[1]*, A. Gaggar*[2]*, G. Tavernier*[3]*, L. G. Gregory*[1]*, A. Simpson*[3]*, C. M. Lloyd*[1]*, R. J. Snelgrove*[1]*;*
[1]*Imperial College London, London, United Kingdom,* [2]*University of Alabama, Birmingham, United States,* [3]*University of Manchester, Manchester, United Kingdom.*

The enzyme leukotriene A_4 hydrolase (LTA_4H) is classically recognized for its capacity to generate the potent pro-inflammatory mediator leukotriene B_4 (LTB_4). However, we have previously demonstrated that LTA_4H exhibits a secondary anti-inflammatory activity whereby it degrades the collagen-derived tripeptide and neutrophil chemoattractant Pro-Gly-Pro (PGP). LTB_4 has been implicated in the pathology of many chronic diseases, with a prominent role ascribed to this mediator in driving inflammation and airway hyperresponsiveness (AHR) in asthma. Accordingly, pharmaceutical companies have developed LTA_4H inhibitors to ameliorate LTB_4-driven pathologies, but these have thus far failed to demonstrate efficacy in the clinic - potentially owing to their inadvertent capacity to cause PGP accumulation. In a house dust mite model of allergic airways disease, we show that specific abrogation of LTB_4 signaling ameliorated inflammation and AHR, yet global loss of LTA_4H exacerbated AHR - despite the absence of LTB_4 and a profound reduction in T_H2 inflammation. This exacerbated AHR was attributable to PGP accumulation and a neutrophil-independent capacity of this peptide to promote pathological airway epithelial remodeling and mucus production- a phenotype recapitulated by direct application of PGP to human airway epithelial cells at air-liquid-interface. Subsequently, we show that PGP accumulates preferentially in the sputum of severe asthmatics in two distinct cohorts of patients. In conclusion, we highlight the capacity of a fragment of collagen to directly drive pathological airway remodeling. These studies have implications for our understanding of remodeling phenotypes in asthma, and other chronic diseases and may rationalize the failure of LTA_4H inhibitors in the clinic.

P.C5.01.18

Active transport of iron-flavonoid complexes by the major allergen Bet v 1 leads to enhanced activation of the aryl hydrocarbon receptor

A. Regner[1]*, M. Czernohaus*[1]*, G. Hofstetter*[1]*, K. Kienast*[1]*, Z. Dvorak*[2]*, L. F. Pacios*[3]*, E. Jensen-Jarolim*[1,4]*, F. Roth-Walter*[1]*;*
[1]*Department of Comparative Medicine, Interuniversity Messerli Research Institute, University of Veterinary Medicine Vienna, Medical University Vienna, University of Vienna, Vienna, Austria,* [2]*Department of Cell Biology and Genetics, Faculty of Science, Palacky University, Olomouc, Czech Republic,* [3]*Center for Plant Biotechnology and Genomics and Department of Biotechnology-Vegetal Biology, ETSIAAB, Technical University of Madrid, Madrid, Spain,* [4]*Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University Vienna, Vienna, Austria.*

Introduction: Our *in silico* calculations predicted that the major allergen from birch, Bet v 1, has lipocalin-like function and is able to bind iron via high-affinity-iron-chelators called siderophores. Only when loaded with iron it was *in vivo* able to prevent to the development of IgE and allergic sensitization. Here we analysed *in vitro* the iron-binding capacity of Bet v 1, using flavonoids (catechol-type-siderophores). Furthermore, we determined the bioactive function of these iron-chelators focusing on the aryl-hydrocarbon-receptor (AhR).
Materials and Methods: UV/VIS-spectra of three major flavonoids (Quercetin, Catechin, Epi-Catechin) in the presence or absence of allergens and iron were analyzed. Using the reporter cell line AZ-AhR activation of the AhR-pathway was determined by measuring luciferase activity. Presence of iron was measured with Calcein.
Results: All three tested flavonoids served as ligands for Bet v 1 regardless of the presence or absence of iron. Binding of these flavonoids alone or in complex with iron to Bet v 1 significantly enhanced AhR-activation in a concentration-dependent manner indicating active shuttling of flavonoids into the intracellular compartment. Moreover, iron transport into cells was confirmed by Calcein measurements.
Conclusions: Flavonoids act as siderophores and bind to Bet v 1. Only the loaded form of Bet v 1 significantly stimulated AhR-activation via active transport of these flavonoids and iron into the cell, thereby enabling an immune-suppressive stimulus. The ligands of allergens may thereby be decisive for the subsequent immune response promoting tolerance.
The study was supported by the Austrian Science Fund FWF, grant SFB F4606-B28 to EJJ.

P.C5.01.19

Olaparib, a Poly(ADP-ribose) Polymerase Inhibitor Abates Ovalbumin-induced Airway Inflammation and Remodeling in Murine Model of Chronic Asthma

G. S. Sethi*, A. S. Naura;*
Department of Biochemistry, Panjab University, Chandigarh, India.

Poly(ADP-ribose) polymerase (PARP) has been reported to play a crucial role in the manifestation of allergen-induced airway inflammation, a characteristic feature of acute asthma. However, the role of PARP in airway remodeling (a hallmark of chronic asthma), is not completely known. Accordingly, the present study was designed to evaluate the potential of olaparib (a PARP inhibitor) on airway remodeling traits using an ovalbumin (OVA)-induced murine model of chronic asthma. The results demonstrated that olaparib administration (5 mg/kg b.wt. *via i.p.*), 30 minutes after every OVA challenge for six-weeks attenuates the airway inflammation, mucus production, collagen deposition in airways and the expression of coupled factors such as Th2 cytokines, Muc5ac, Col1α1, MMP-9, and TGF-β in lung tissues. Additionally, the OVA-induced alteration in the level of ROS, MDA, protein carbonyls and GSH/GSSH ratio were restored toward normal upon olaparib treatment. Furthermore, drug suppressed the expression of STAT-6, GATA-3, and vimentin, markedly along-with the partial attenuation of NF-κB activation. Overall, our results suggest the beneficial potential of olaparib in the prevention of airway remodeling associated with OVA-induced chronic asthma in mice.

P.C5.01.20

Allergic sensitization profile of polysensitized asthmatic patients in Southern China using molecule-based IgE technique

H. Hu, ***B. Sun****, W. Luo;*
First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China.

Objective: Most of allergic asthma patients are sensitization to a variety of allergens. This study aimed to analyze the sensitization profiles of polysensitized asthmatic patients in southern China utilizing molecule-based IgE diagnostic technique. **Method:** Serum samples from 63 asthma patients in southern China were tested with ISAC for specific immunoglobulin E (sIgE) against 112 single allergen components. **Results:** In this group of patients, 79.36% showed sIgE positive to more than three allergen components. Polysensitized asthmatic patients in southern China were mainly allergic to rDer f 2 (68.25%), nDer f 1 (66.67%), nDer p 1 (65.08%), rDer p 2 (61.90%), rFel d 1 (26.98%), nCyn d 1 (15.87%), nPhl p 4 (14.29%), rCanf 1 (12.70%), rDer p 10 (11.11%) and rLep d 2 (11.11%). Polysensitized asthma patients complicated with rhinitis showed higher positive rates of the allergen components Phl p 4 (19.05% vs. 0.00%) and nCyn d 1 (26.19% vs. 4.76%) than patients without rhinitis ($P < 0.001$). Among food allergen components, the walnut allergen component nJug r 2 showed the highest positive rate (9.52%). An optimal scaling analysis indicated that a positive test of rDer p 10 was associated with food allergy (Cronbach's Alpha = 92.0%).**Conclusions:** The sensitization profiles of polysensitized asthmatic patients in southern China were different from other countries. Polysensitized asthma patients complicated with rhinitis showed higher positive rates for nPhl p 4 and nCyn d 1 than without. Polysensitized asthmatic patients who positive for rDer p 10 were associated with food allergy.

P.C5.01.21

The value of allergy screening in Chinese adult patients with chronic respiratory diseases

*H. Hu, **B. Sun**, P. Zheng;*
First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China.

Objective: The prevalence of allergen-induced chronic respiratory disease (CRD) is increasing annually. This study aimed to analyze sensitization characteristics of adult Chinese CRD patients and the value of allergy screening. **Methods:** Serum immunoglobulin E (sIgE) was detected with an allergy screening test. Total immunglobulin E (tIgE) levels were measured in 85 asthma patients, 98 chronic obstructive pulmonary disease (COPD) patients, and 69 patients with other CRDs. **Results:** The total positive rate of allergy screening among CRD patients was 36.1%. Asthma had the highest rate (45.9%), followed by COPD (32.7%) and other CRDs (29.0%). The positive rate of allergy screening was significantly higher among urban asthma patients (56.1%) than among rural patients (25.0%, $P < 0.05$), and significantly higher among office staff (68.9%) than among outdoor workers (42.8%, $P < 0.05$).
Patients with COPD (42.9%) and recurrent exacerbation were atopic. Atopy was a risk factor for dyspnea (OR = 1.22; 95% CI, 0.95-1.75, $P < 0.05$).
Optimal scaling analysis revealed a correlation between tIgE levels and smoking index (Cronbach`s alpha = 91.1%). Up to 35.0% of patients who met the Global Initiative for Chronic Obstructive Lung Disease III (GOLD III) IV criteria and had a low Phadiatop level (≤10 kU/L), had a high tIgE level (≥1000 kU/L), compared to GOLD I II patients (5.5%). **Conclusion:** Patients with CRD had high sensitization. Asthma patients who work indoors were more susceptible to allergies. Atopy was associated with COPD pulmonary function. It is necessary to initially screen the sensitization situation of CRD patients.

P.C5.01.22

Maternal IgG impairs the maturation of offspring intrathymic IL-17-producing γδT cells: possible implications for murine and human allergy

*M. G. de-Oliveira[1], A. A. Lira[1], F. R. Sgnotto[1], A. H. Inoue[1], L. S. Santos[1], A. J. Duarte[1], M. Leite-de-Moraes[2], **J. R. Victor**[1,3];*
[1]School of Medicine - USP, Sao Paulo, Brazil, [2]Institut Necker-Enfants Malades (INEM), Paris, France, [3]Laureate International Universities (FMU), São Paulo, Brazil.

Using a well-standardized murine model of offspring allergy inhibition mediated by maternal allergen immunization, we aimed to evaluate the relationship between IL-17-producing γδT cells and allergy inhibition by focusing on the regulation of the intrathymic maturation and γδT cell biology. Female mice were immunized or not, and the allergic response, frequency of γδT cell subsets and cytokine production of the offspring were analysed by flow cytometry. The effects of passive in vivo transfer of thymocytes or purified IgG were investigated in offspring. A translational approach was employed to analyse γδT cells in the thymus and PBMCs from humans. Maternal immunization reduced the frequency of spontaneous IL-17-producing γδT cells in the thymus, spleen and lung of offspring. This effect was mimicked by the in vivo treatment of females with purified IgG. IgG directly interacted with γδT cell membranes. Human infant intrathymic γδT cells showed reduced IL-17 production in response to purified IgG from non-atopic individuals, whereas adult peripheral γδT cells from atopic individuals were prone to produce IL-17 in response to IgG. Together, our results reveal that IgG from atopic mice can influence the thymic γδT cell maturation. Further, IgG is an unprecedented modulatory factor of murine and human γδT cells.

P.C5.01.23

Mango chitinase is a major allergen in Chinese pediatric patients

***L. Xia**[1], H. Cao[1], X. Xu[1], X. Xiao[1], D. Cai[2], Z. Chen[3], P. Yang[1], Z. Liu[1];*
[1]College of Medicine, Shenzhen University, Shenzhen, China, [2]Clinical molecular diagnostic laboratory, Shenzhen Children's Hospital, Shenzhen, China, [3]Department of Pediatrics, Third Affiliated Hospital of Sun Yat-sen University, Shenzhen, China.

Introduction: Allergies to mango are frequently observed in clinical practice. However, only one allergen has been identified from mango until now.Materials and Methods: Protein crude extract of mango flesh was resolved by using 1-D SDS-PAGE. The reactive bands were analyzed by MS/MS mass spectrometry. A band showed significant homology to the mango chitinase (Genbank Accession: ACD69683.1). The cDNA of the chitinase was synthesized and cloned into a plasmid pMAL-C5X and expressed as a recombinant protein. The reactivity of the recombinant protein towards mango allergic patients was assayed by using western blot. The linear IgE epitopes of the chitinase were then analyzed by six peptides spanning the entire protein with putative potential of IgE reactivity. Results: The mango chitinase reacted with IgE of 9 out of 13 sera (69%) of pediatric patients with mango allergies. Only one peptide (8-28) which contains the chitin-binding domain possesses IgE reactivity. Conclusions: The mango chitinase is a major allergen in Chinese pediatric patients.

P.C5.02 Allergy, asthma and therapy - Part 2

P.C5.02.01

The skin as a mirror of the functionality of our immune system

L. N. Ahmetaj;
University of Prishtina, Prishtina, Kosovo, Republic of.

Case report

Woman I.M., 1950, Prishtina,with ambulantory diagnosis Dermatitis cruris, Erythema region faciei. The patient showed up to our hospital with multiforme erythematous changes along crural and palmoplantar region, following unsuccessful five-month ambulantory treatment. During physical examination, we observed asymmetrical erythematous changes in both legs and palmoplantar regions, with induration, without pain. Laboratoric data showed high level of erythro-sedimentation 30/66, RBC=5.21, PLT =246,Le=6.4.
Chemical analyses were normal (urea,creatinine, bilirubin, ALT and AST)
Protein C reactive= 2.40(0.6),Rheumatoid factor 12.0(<12) andASTO=400 IU/ml (200 IU/ml)
Normal Rtg pulmis.
In alergologic tests the level of total IgE is normal, eosinophilia (4%), positive skin prick test in D.pteronyssinus and D.farinae (house dust mites). We concluded with erythema multiforme as a diagnosis and started with conservative anti-allergic treatment with corticosteroids and antihistamines and also antibiotics. Etiological treatment continues with subcutaneous specific immunotherapy to house dust mites. After a five-year treatment,we consider that our patient is cured. But, there were some unusual findings in this case: Unusual in this case was that the EM is described as allergic reaction with unknown etiology, type IV (four). Different allergic triggers in this condition are prescribed (viral infections, bacterial, mycotic,etc).Histologically, IgM depositions were found along small blood vessels.
On the other side, we know that specific immunotherapy is an etiological treatment for IgE mediated allergic reactions.

P.C5.02.02

Cyclic-dipeptide isolated from *Hirsutella sinensis*mycelium attenuates ovalbumin-induced asthma in a murine model

***L. B. Antig**[1], C. Lin[1,2], Y. Chen[3], P. Hsieh[3], Y. Ko[4], H. Lai[1,2];*
[1]Department of Medical Biotechnology and Laboratory Science, Chang Gung University, Taoyuan, Taiwan, [2]Microbiota Research Center, Center for Molecular and Clinical Immunology, Chang Gung University, Taoyuan, Taiwan, [3]Graduate Institute of Natural Products, Chang Gung University, Taoyuan, Taiwan, [4]Chang Gung Biotechnology Corporation, Taoyuan, Taiwan.

Background: Asthma is a chronic inflammatory disorder that affects millions of people worldwide. It is characterized by eosinophilic inflammation, airway hyperresponsiveness, and airway remodeling. Traditional Chinese Medicine has been used for centuries for their immunomodulatory and anti-inflammatory effects. *Hirsutella sinensis*, a medicinal mushroom, was found to have anti-asthmatic effects. Water extracts from *Hirsutella sinensis* mycelium reduced ovalbumin-induced asthma and TH2 responsiveness in mice, yet the bioactive compound that promotes this occurrence remains unknown.
Materials and Methods: Bioactivity-graded fractionation such as thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) and an *in vitro* TH2 cell inhibition screening model was used to isolate and identify the cyclic-dipeptide from *Hirsutella sinensis* mycelium. Ovalbumin-induced asthma model in BALB/c mice were utilized to test the compound *in vivo.*
Results: Cyclic-dipeptide directly inhibited TH2 responsiveness by decreasing production of TH2-associated cytokines such as IL-4, IL-5, and IL-13 *in vitro* and *in vivo.* Further *in vivo* asthma model demonstrates that cyclic-dipeptide from *Hirsutella sinensis* mycelium dose-dependently attenuates ovalbumin-induced asthmatic responses, including airway hyperresponsiveness, immune cells infiltration, TH2 cytokines responses, and ovalbumin-specific humoral responses.
Conclusions: Taken together, we identified a patentable anti-asthmatic and anti-allergic compound isolated from HSM and potentially explore its underlying mechanisms.

P.C5.02.03
Effects of Immunotherapy on different allergic conditions

F. Arooj;
Sir Gangram hospital, lahore, Pakistan.

Background: The study was conducted in Sir Ganga Ram Hospital. 100 patients were randomly selected and their allergy status and the effects of immunotherapy were determined. 36% had bronchial asthma, 32% had allergic rhinitis, 18% had allergic conjunctivitis, 10% had urticaria and 4% had atopic dermatitis.
Methods: Provisional diagnosis of allergic symptoms was confirmed by the skin prick test, before and after immunotherapy, against various pollens (mixed trees, weeds, sunflower [Helianthus annuus], Acacia, bottlebrush [Elymus elymoides]), dust, paper mulberry and dandelion [Taraxacum officinale]. Aqueous mixed allergen preparations were used for immunotherapy. The 1st injection administered was a small dose (0.5 ml), and gradually increased to 1 ml, from a low concentration to a higher concentration, over a period of 3 months. Immunotherapy lasted for 1 year. A placebo control study was also conducted simultaneously on 50 patients selected randomly in the same manner.
Results: The results clearly indicated that immunotherapy was a useful treatment against all allergic diseases, with varying effectiveness. Percentage improvement was highest (73.75%) for allergic rhinitis in the 26-40 years age group and lowest (20.00%) for urticaria in the 10-25 years age group. Therapy was also found to be useful in all other age groups.
Conclusions: The study revealed that the various grass pollens and dust particles gave the highest degree of allergic reaction in the patients.

P.C5.02.04
The quest for bacterial allergens - Serine proteases of *Staphylococcus aureus* are inducers of type 2 airway inflammation

*M. Nordengrün[1], A. Teufelberger[2], S. Michalik[3], S. Stentzel[1,4], F. Schmidt[3], C. Bachert[2], U. Völker[3], O. Krysko[2], **B. M. Bröker**[1];*
[1]Department of Immunology, University Medicine, Greifswald, Germany, [2]Upper Airways Laboratory, Ghent University, Ghent, Belgium, [3]Department of Functional Genomics, University Medicine, Greifswald, Germany, [4]IDT Biologika, Greifswald-Insel Riems, Germany.

Introduction: According to the hygiene hypothesis, childhood infections as well as early exposure to microbial diversity protect from allergy. *Staphylococcus aureus*, in contrast, is associated with allergic reactions, besides its well-known commensal and invasive behavior. In patients suffering from allergic airway inflammation, *S. aureus* colonization is much more frequent than in controls. However, the driving allergens of *S. aureus* remained elusive.
Methods and Results: By immunoproteomics we identified the serine protease-like proteins (Spls) A-F as major IgG4-binding proteins of *S. aureus*. IgG4 served as a surrogate marker for IgE, since production of IgG4 and IgE are both initiated by a similar Th2 cytokine profile. The *S. aureus* Spls A-F are extracellular proteases of so far unknown function. We observed increased Spl-specific serum IgE titers in asthma patients. Following stimulation with Spls, memory T cells of healthy donors secreted Th2 cytokines. In contrast, Th1/Th17 cytokines were of low concentration or absent. In mice, intra-tracheal application of SplD without adjuvant induced allergic lung inflammation, including infiltration of inflammatory cells and generation of SplD-specific serum IgE. This identifies SplD as a triggering allergen of *S. aureus*. To elucidate the underlying mechanism, we blocked the downstream effects of IL-33 by co-administration of sST2, the soluble IL-33 receptor. sST2 treatment counteracted the SplD-mediated allergic lung inflammation, indicating that SplD causes a Th2 response in an IL-33 dependent manner.
Conclusion: We identified Spls as triggering allergens of *S. aureus,* opening prospects for diagnosis and causal therapy of asthma.

P.C5.02.05
Inhibition of basophil activation by an Omalizumab-resistant IgE-Fc glycovariant

*P. Gasser[1], L. Pennington[2], D. Brigger[1], N. Zbären[1], T. Jardetzky[2], **A. Eggel**[1];*
[1]Department for Rheumatology, Immunology and Allergology, 3010 Bern, Switzerland, [2]Department of Structural Biology, Stanford University School of Medicine, Stanford, California 94305, United States.

Background: Allergen-specific IgE plays a major role in immediate hypersensitivity reactions. It binds with high-affinity to its primary receptor FcεRI on basophils and mast cells. Upon allergen stimulation, these cells degranulate and release soluble mediators causing allergic symptoms. The therapeutic anti-IgE antibody Omalizumab is known to prevent binding of IgE to basophils and mast cells. Recently, we have reported that Omalizumab actively desensitizes basophils at high concentrations. Furthermore, we have provided evidence that an IgE-Fc glycovariant, which is resistant to Omalizumab binding, may be used to replace the IgE-repertoire on the surface of primary human basophils when co-applied with Omalizumab. This combination treatment significantly increased inhibition of antigen-mediated basophil activation ex vivo. Here, we provide mechanistic insight into the mode of action of this IgE-Fc glycovariant.
Methods: Human primary basophils were isolated from whole blood donations of grass-pollen allergic individuals and treated with the IgE-Fc glycovariant alone or in combination with Omalizumab. Subsequently, cells were challenged with a grass-pollen allergen mix. Basophil activation was measured by flow cytometry.
Results: Interestingly, the IgE-Fc glycovariant alone diminished basophil activation in a competition-independent manner. Furthermore, it showed dose-dependent inhibition already at low concentrations when used in combination with Omalizumab.
Conclusions: Our data indicate that the IgE-Fc glycovariant induces inhibitory signaling in human primary basophils. The IgE-Fc variant could potentially be used as an efficient add-on treatment to the current Omalizumab therapy.

P.C5.02.06
Modulation of allergen-specific immune response with allergen-laden virus-like nanoparticles (VNP) co-expressing surface-anchored cytokines and growth factors

***S. Hofer**, B. Kratzer, D. Trapin, W. F. Pickl;*
Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria.

The expression in producer cells of structural core proteins of *Moloney* murine leukemia virus (MoMLV) leads to the formation of non-infectious virus-like nanoparticles (VNP), which become enveloped by the lipid-raft containing regions of the plasma membrane through which they are budding. This had allowed to decorate VNP with a collection of immunomodulatory and fluorescent molecules by their fusion to lipid modification sequences or viral structural proteins in the past. Here we examined the immunomodulatory potential of surface-anchored cytokines on VNP co-expressing the major mugwort pollen allergen, Art v 1. The coding sequences of GM-CSF, IFN-γ, IL-10, IL-12 and TGF-β1 were C-terminally fused to the GPI anchor acceptor sequence of CD16b and tested for surface expression on producer cells and VNP. Subsequently, they were evaluated for their potential to modulate effector functions of allergen-specific T cells from humanized mice which are reactive to mugwort pollen allergen. All five GPI-anchored fusion proteins expressed well on producer cells and VNP alike, as confirmed by flow cytometry and biochemical analyses. Notably, the expression of the different cytokines and growth factors co-stimulated the proliferation of allergen-specific $CD4^+$ Teff cells towards the allergen, with the exception of IL-12, which inhibited proliferation by 50 percent when compared to VNP decorated with allergen alone. Notably, IL-12 decorated, allergen-expressing VNP strongly induced IFN-γ expression in $CD4^+$ Teff cells, which, however, elaborated 20-30-fold less Th2 and Th17 cytokines. Cytokine decorated, allergen-specific VNP might help prevent and treat allergies in the future. Supported by Austrian Science Fund (FWF) DK-W 1248, SFB-F4609.

P.C5.02.07
Asthmatic airway inflammation is alleviated by adeno-associated viral vectors carrying CD39 in OVA-sensitized mice model

***Y. Huang**[1], C. Kuo[1], J. Ma[1], M. Kuo[1,2];*
[1]Graduate Institute of Biomedical Sciences, Chang Gung University, Taoyuan City, Taiwan, [2]Department of Microbiology and Immunology, Chang Gung University, Taoyuan City, Taiwan.

Introduction: Asthma is a chronic respiratory disease characterized by recurrently attacks of breathlessness and wheezing. ATP accumulation in bronchoalveolar lavage fluid (BALF) of asthmatic subjects indicates that extracellular ATP (eATP) and downstream responses are involved. The level of eATP is tightly controlled by apyrase (also named as CD39) in healthy tissues and it is downregulated upon inflammatory stimulation. In order to transduce exogenous genes, recombinant adeno-associated virus (rAAV) has been developed and characterized by low toxic and difference tissue tropisms among serotypes.
Materials and Methods: ATP levels in supernatants of stimulated RAW 264.7 cells and BEAS-2B cells were determined. rAAV-CD39 was generated and applied to OVA-mediated asthmatic mice model. The mice were sensitized intraperitoneally and challenged intratracheally with OVA and treated with rAAV-CD39 three days before OVA challenge. At the end of procedure, some asthmatic and inflammatory cardinal features were examined.
Results: Elevated ATP level was determined either in stimulated-culture supernatant and BALF of OVA-sensitized mice. rAAV-CD39 treatment downregulated the level of ATP in BALF with rescued expression of *cd39*. Several asthmatic features, such as eosinophilia, IL-13 production from draining lymph node cells and AHR were decreased in the rAAV-CD39 treated group. The frequency of $CD4^+FoxP3^+$ regulatory T cells was also increased in lymph nodes. However, OVA-specific IgE and IgG1 were not impacted.
Conclusion: The treatment of rAAV carrying CD39 attenuated the asthmatic airway inflammation locally. We consider that extracellular ATP signaling could be a potential target for gene therapy on asthma.

P.C5.02.08

Intranasal inoculation of PM2.5 induces inflammation in both the lung and liver of the mouse via distinct signaling pathway

S. Jeong[1], *S. Park*[1], *I. Park*[2], *P. Kim*[2], *N. Hoon*[3], *Y. Hyun*[1];
[1]*Department of Anatomy and Brain Korea 21 PLUS Project for Medical Science, Seoul, Korea, Republic of,* [2]*Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of,* [3]*Department of Pathology, Yonsei University College of Medicine, Seoul, Korea, Republic of.*

Particulate matter (PM2.5) is currently major issue of air pollutants to threat public health related to the exposure through pulmonary pathway. Although many studies have been performed to directly treat mouse model with PM2.5, there were few reports about PM2.5 using two-photon intravital imaging. In this study, we aimed to investigate neutrophil migratory pattern and structural changes in the lung and liver, respectively. To compare the inflammatory response of both organs, we performed two-photon intravital imaging of mice lung and liver, respectively at 24 and 48 hours post to PM2.5 treatment through intratreacheal inoculation. We found that there was no significant difference in the number of neutrophils in both lung and liver of PM2.5-treated mice compared to the control mice. On the other hand, using immunohistochemistry and electron microscopy, we found that lymphocyte number was significantly higher in the lung and liver of PM2.5 treated mice than the control mice. Also, from the microarrays of PM2.5-treated organs of mice, there were distinct upregulations of mRNA in the lung and liver, respectively, in response to PM2.5 treatment. While lung showed the upregulation of mRNAs associated with IL-17 signaling pathway related to the regulation of neutrophil, liver showed high level expression of mRNAs associated with metabolic pathways. Therefore, these results suggest that lung is distinctly regulated from liver under acute PM2.5-induced inflammatory condition. In summary, our data of lung and liver of PM2.5-treated mice uncovered that lung and liver are regulated via distinctive signaling pathway.

P.C5.02.09

Interleukin 2 helps to detect the allergen responsive lymphocytes in whole blood cultures

P. Jinoch*, M. Prouza;*
EXBIO Praha, Vestec, Czech Republic.

Ki-67 protein was reported as a quantitative indicator of lymphocyte proliferation in PHA stimulated cultures. Its detection was shown to be more sensitive than the DNA content analysis and the measurements of incorporated nucleoside analogues. We used Ki-67 staining in whole blood cultures stimulated with food allergen extracts to evaluate specimens obtained from patients previously diagnosed with delayed type of hypersensitivity to gluten. Allergen extracts were aseptically lyophilized at the bottom of polystyrene tubes with dual position caps. The tubes were intended to the cell culture, antibody staining and cytometry analysis. Whole blood was diluted 1:10 in culture medium and cultivated for 7 days. Various amounts of interleukin 2 were added to the medium to improve the sensitivity of the test. Tetanus vaccine was used as the positive control. Samples were stained with CD3/CD4/Ki-67. The flow cytometry analysis of unstimulated lymphocytes revealed virtually no background. The Ki-67 antibody conjugate to phycoerythrin provided high signal to noise ratio thus the previously described variations in Ki-67 expression were negligible in discrimination between negative and positive cells. All the cultures supplemented with interleukin 2 were found responsive for the respective allergen with clear dose-response curves. The blood stimulations without interleukin 2 were negative in allergen stimulations and sometimes in tetanus stimulations. We conclude that supplementation with interleukin 2 helps to reveal the presence of allergen specific lymphocytes that would otherwise be missed in whole blood allergen specific stimulations.

P.C5.02.10

NK-cell mediated contact hypersensitivity in obese mice

P. Kowalczyk*, D. Biała, A. Strzępa, K. Marcińska, M. Majewska-Szczepanik, M. Szczepanik;*
Department of Medical Biology, Faculty of Health Sciences, Jagiellonian Univeristy Medical College, Krakow, Poland.

Previous experiments showed that liver NK cells can mediate contact hypersensitivity (CHS) reaction in mice. To investigate influence of diet-induced obesity on NK cell-mediated CHS, RAG1-/-B6 mice were maintained on HFD or normal diet (ND). After 8 weeks the animals kept on HFD or ND were split into two groups, one of which was sensitized with DNFB, while the second one was treated with solvent alone. Then, the animals were challenged with DNFB on the ears and tested for the CHS reaction. The subcutaneous fat tissue was collected and weighed and then cells obtained by mechanical dissociation were cultured to determine cytokine production. Additionally, lipid profile in HFD and ND mice was determined. Also, liver mononuclear cells (LMNC) were cultured with DNP-BSA antigen to determine antigen-specific cytokine production. We found that mice fed HFD for 8 weeks developed aggravated CHS reaction determined by ear swelling measurement when compared to animals kept on ND prior to CHS sensitization and challenge. The increased ear swelling in HFD-fed mice was confirmed by increased MPO activity in ear homogenates, higher ear weight and increased vascular permeability. Subcutaneous fat tissue from mice on HFD weighed more and produced more IL-6 but less IL-10 when compared to ND mice. RAG1-/-B6 mice on HFD showed higher concentration of total cholesterol and LDL-cholesterol in serum when compared to ND mice. Finally, LMNC from HFD mice restimulated *in vitro* with DNP-BSA produced more IFN-γ than LMNC from ND mice. Supported by grant from National Science Center 2016/23/N/NZ6/01488 to PK

P.C5.02.12

Overexpression of the AP-1 subunit, Fra2 causes a non-allergic asthma phenotype in mice

L. M. Marsh[1], *A. Gungl*[1,2], *V. Biasin*[1], *J. Wilhelm*[3,4], *G. Kwapiszewska*[1,2];
[1]*Ludwig Boltzmann Institute for Lung Vascular Research, Graz, Austria,* [2]*Otto Loewi research center, Medical University of Graz, Graz, Austria,* [3]*Department of Internal Medicine, Universities of Giessen and Marburg Lung Center, Giessen, Germany,* [4]*German Center for Lung Research, Justus-Liebig University, Giessen, Germany.*

Introduction: Asthma is a complex chronic inflammatory disease characterised by airway inflammation, remodelling and hyperresponsiveness (AHR). Members of the AP-1 family of transcription factors have been shown to play important roles in the activation of the immune system and the control of cellular responses. Here we have investigated the role of the lesser known AP-1 family member, Fra2 in experimental asthma.
Methods and Results: Gene expression profiling of Fra2 overexpressing (TG) mice revealed a high number of regulated genes associated with airway remodelling, inflammation and mucus production. In line with this finding TG mice exhibited increased airway remodelling, with peribronchial collagen and smooth muscle thickening as well as mucus production. TG mice possessed a strong inflammatory infiltrate in the lung, predominately, eosinophils and T cells and increased expression of Th2 cytokines and eotaxin. Furthermore, TG mice possessed AHR in response to increasing doses of methacholine. Therapeutic intervention via IL-13 blocking antibodies or corticosteroids partially reduced AHR, mucus secretion and eosinophil recruitment. However, only corticosteroid treatment could reduce all aspects of airway remodelling.
Conclusion: Here we describe a novel model of non-allergic asthma, which does not require the application of exogenous allergens. Fra2 represent a key molecule that coordinates several aspects of asthma pathogenesis, including airway inflammation, remodelling and hyperresponsiveness.
This study was funded in part by the Austrian Science Fund (FWF): P27848-B28 and Austrian National Bank grant number 16187.

P.C5.02.13

Quality of life in patients with bronchial asthma during immunotherapy with autologous activated T-lymphocytes

E. Pashkina*, A. Makarova, E. Blinova, V. Nepomnyashchikh, M. Leonova, D. Demina, V. Kozlov;*
Research Institute of Fundamental and Clinical Immunology, Novosibirsk, Russian Federation.

Today bronchial asthma (BA) is one of the most common chronic diseases associated with immunological disorders. The purpose of this study was to study the effect of immunotherapy with activated autologous T-lymphocytes, including the evaluation of the quality of life in the dynamics of this type of therapy in patients with BA.
The study included 23 patients, (average 38.5 ± 4.3 years) with moderate severity of BA, receiving treatment at the Clinic of Immunopathology of RIFCI, Novosibirsk. The study was approved by the ethical commission of RIFCI. After obtaining Informed Consent to participate in the study, patients were injected subcutaneously with autologous activated T cells according to scheme: 1 injection once a week - 4 times, then 1 injection per month - 6 times. Patients also received basic therapy. A study of quality of life in patients was carried out using the AQLQ(S). The AQLQ(S) has 4 domains: activity limitations, symptoms, emotional function, and exposure to environmental stimuli. After 2 months, it was observed significant improvement in the “activity limitations” domain compared with the values before the beginning of therapy. Also there was a tendency (p=0.08) to increase the indicators in the domain “emotional function”. After 7 months, in the end of immunotherapy, it was shown a statistically significant improvement in all domains of AQLQ(S) compared before treatment. That was indicated the efficacy of the immunotherapy.
Our findings suggest that the therapy with autologous activated T-cells led to an improvement in the quality of life in patients with BA.

P.C5.02.14

Tolerogenic Immune Modifying Nanoparticles as an Antigen-Specific Therapy for the Treatment of Th2-Mediated Food Allergy

J. R. Podojil[1,2]*, C. Smarr*[1]*, T. P. Neef*[1]*, R. M. Pearson*[3,2]*, D. Getts*[2]*, P. J. Bryce*[1]*, L. D. Shea*[3]*, S. D. Miller*[1]*;*
[1]Northwestern University, Chicago, United States, [2]COUR Pharmaceuticals Development Company, Chicago, United States, [3]University of Michigan, Ann Arbor, United States.

Standard allergy treatments fail to target the underlying Th2-mediated cause of disease. Thus, development of safer, more efficient means of inducing antigen-specific tolerance is a major goal of allergic disease research. Previously published data show that i.v. injection of antigen-pulsed splenic antigen-presenting cells (Ag-SP) chemically fixed with ethylene-carbodiimide (ECDI) safely inhibit Th1/Th2/Th17-mediated disease via the induction of antigen-specific tolerance. We hypothesized that Tolerogenic Immune Modifying Nanoparticles (TIMP; poly(lactide-co-glycolide nanoparticles containing an encapsulated disease-associated antigen), which are in clinical trial for treating celiac disease, would also be effective for the induction of immune tolerance in murine models of Th2-mediated food allergy. Importantly, the treatment of WPE/alum primed mice with TIMP-WPE has been shown to be safe when administered via i.v. injection. Additionally, the present data show treatment of mice with TIMP-WPE either prophylactically or therapeutically in WPE-induced food allergy resulted in a decrease in the level of IL-4, IL-5, and IL-13 secreted upon ex vivo recall culture of splenocytes. Thus, we illustrate progression toward the safe and effective inhibition of food allergy without the need for nonspecific immunosuppression in animals with established Th2 sensitization.

P.C5.02.15

The role of atopy in the pathogenesis of bleomycin induced lung fibrosis

L. Pur Ozyigit[1,2]*, E. Cetin Aktas*[3]*, Z. Senbas*[4]*, A. Ozturk*[5]*, E. Ozturk*[6]*, M. Ergonul*[7]*, L. Tabak*[8]*, B. Ferhanoğlu*[9]*, M. Cetiner*[9]*, G. Deniz*[3]*;*
[1]Koç University, School of Medicine, Department of Allergy and Immunology, Istanbul, Turkey, [2]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Immunology Department, Istanbul, Turkey, [3]Istanbul University, Aziz Sancar Institute of Experimental Medicine, Immunology Department, Istanbul, Istanbul, Turkey, [4]Koç University, School of Medicine, Istanbul, Turkey, [5]Koç University Hospital, Department of Allergy and Immunology, Istanbul, Istanbul, Turkey, [6]Koç University Hospital, Department of Haematology, Istanbul, Istanbul, Turkey, [7]Koç University, School of Medicine, Department of Infectious Diseases, Istanbul, Istanbul, Turkey, [8]Koç University, School of Medicine, Department of Respiratory Medicine, Istanbul, Istanbul, Turkey, [9]Koç University, School of Medicine, Department of Haematology, Istanbul, Turkey, Istanbul, Turkey.

Bleomycin pulmonary toxicity (BPT) is a potentially life-threatening consequence of bleomycin usage in patients. An overproduction of epithelium-derived cytokines, habitually linked to allergic inflammation, has been recently revealed in experimental models of BPT. We assessed retrospectively our cohort of patients with Hodgkin Lymphoma treated with bleomycin between 2014-2016 for their demographic, clinical features, including BPT development, atopy status and risk factors for BPT. Then they were invited for allergy testing and blood sample collection. The samples were stimulated with different stimuli (Bleomycin, IL-33, TSLP) for 24 hours *in vitro* cell culture. The culture supernatants were analysed for TGF-β, Galectin3, Arginin, Amphiregulin, Eotaxin, IFN-γ, TNF-α, IL1-β, -4, -5, -6, -10, -13, -17, MIP-1α and bleomycin hydrolase (BLH) levels by ELISA and LUMINEX. The cohort consisted of 51 patients showed that atopy was the only significant risk factor for BPT occurrence (OR: 7.2, p=0.007). Fourteen subjects were included for blood analysis. The analysis of supernatants at the unstimulated condition revealed that BLH and Amphiregulin were significantly lower in patients who had BPT than controls. The BLH cut-off that best identified a history of BPT was 175.31 (sensitivity: 62.5%, specificity: 100%). Following the stimulation, BLH reduced compared to the unstimulated condition and the difference between groups remained significant (p< 0.05). Our study is the first report that low levels of bleomycin hydrolase in allergic individuals might be predisposing to a possible pathway of fibrosis. This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK), (Grant No: 116S019).

P.C5.02.16

Profile of component-resolved diagnostics allergy by microarray assay in a souther Spanish area

M. San Jose-Cascon*, M. Vilches-Moreno, D. Garcia-Cuesta, A. Sampalo;*
UGC Hematology, Immunology and Genetics, Hospital Universitario Puerta del Mar, Cadiz, Spain.

Identification of sensitization profiles in our geographic area by analysis of component-resolved diagnosis allergy in multiplex support for detection of relevant allergen sources, differentiation between genuine and cross-reactive sensitizations, and analysis of risk and severity of allergic reactions.
160 Patients (72 male/88 female), median aged 31.3 (SD 16.4, range 2-74 years) with suspected allergic symptoms were selected for testing IgE antibodies by microarray assay (ISAC). 74% of the patients showed two or more clinical manifestations at respiratory, digestive and skin levels. 26% of the patients studied showed only one symptom. Unexplained anaphylaxis was present in 62 patients.
ISAC analysis showed sensitization in 131 patients (82%). Sensitization were not found in 29 cases (18%). 87patients (54%) were positive for both, genuine and cross-reactive allergens. Main specific primary sensitization components in respiratory allergy were: ole e 1 (56%), Derp1 (33%), Derp2, Phlp1 (37%), Phlp5 (8%), Alta1 (17%), Salk1 (13%), Artv1 (7%). Primary sensitization components in digestive allergy were nuts: Jugr2 (15%), Jugr1 (11%), Arah1 (4%) and Cora9 (5%) and fruits: Prup1 (5%), Actd1 (6%). Main reactions detected in protein family were: storage protein (49%), nLTPs (47%),lipocalins (21%), Tropomyosins (16%), Profilins (11%), PR10 protein (7%) and polcalcin (3%).
Retrospective analysis of component resolved diagnosis allergy (CRD) by multiplex platform provides useful information about allergenic sources, genuine and secondary sensitization and risk and severity of allergic reaction. A specific map of sensitization in our geographic area will be shown.

P.C5.02.17

IL-33 cooperates with TL1A for potent induction of IL-9-producing ILC2s in allergic airway inflammation

P. Schmitt*, A. Duval, S. Roga, M. Camus, O. Burlet-Schiltz, A. Gonzalez-de-Peredo, C. Cayrol, J. Girard;*
Institut de Pharmacologie et de Biologie Structurale, Toulouse, France.

IL-9 is key driver of chronic and allergic inflammation at mucosal surfaces, with important roles in the activation and survival of mast cells and ILC2s. *IL-9*-reporter mice have established T cells (Th9) and ILC2s as major sources of IL-9 *in vivo*. The discovery that TGF-β and IL-4 cooperate for induction of Th9 cells has been an important breakthrough. However, the signals that induce IL-9+ ILC2s remain incompletely characterized. Here, we show that IL-33, a critical activator of ILC2s, with important roles in type-2 innate immunity and allergic inflammation (Cayrol, Duval and al., Nature Immunology 2018*)* synergizes with TNF-family cytokine TL1A to induce prodigious amounts of IL-9 secretion by ILC2s. Unbiased global proteomic analyses revealed that IL-9 was the most-induced protein in ILC2s treated with IL-33 and TL1A. More than 99 % of ILC2s expressed IL-9 intracellularly after co-stimulation with IL-33 and TL1A. The amounts of IL-9 produced by ILC2s in response to IL-33/TL1A were significantly higher than those that have been reported for Th9 cells cultured with TGF-β and IL-4. ILC2s are known to produce very large amounts of IL-5 and IL-13, but ILC2s producing such high levels of IL-9 have not been described previously. Interestingly, IL-9 production by ILC2s was transient and associated with a phenotypic shift characterized by upregulation of IRF4, pSTAT5 and downregulation of ICOS, KLRG1, and GATA-3. Finally, IL-33 and TL1A expanded IL-9^{high} ILC2s in the lungs and adoptive transfer experiments revealed that IL-9^{high} ILC2s are potent inducers of allergic airway inflammation *in vivo*.

P.C5.02.18

A child with influenza vaccine-associated anaphylaxis without egg allergy

D. Shim*, M. Kim, S. Kim, I. Sol, Y. Kim, K. Kim, J. Lee, M. Sohn;*
Yonsei University College of Medicine, Seoul, Korea, Republic of.

Anaphylaxis associated with influenza vaccines in patients without egg allergy was very unlikely. We reported a pediatric case of influenza vaccine-associated anaphylaxis without egg allergy. A 3 year old girl was admitted to emergency room, showing rash and edema on face, abdominal pain, and hypotension with tachycardia. She was vaccinated with a seasonal quadrivalent influenza vaccine, within 15 hours before onset. She had no history of any allergy, but only had an experience of urticaria after administration of trivalent influenza vaccine last year. Even though she was treated with fluid therapy and epinephrine injection, blood pressure and pulse rates were unstable. She was transferred to PICU for treatment with monitoring. After her vital signs were stable and symptoms were relieved, she could be ward out on the next day. Laboratory findings including allergy test were unremarkable. We collected blood samples on days 1, 3, 7 after anaphylaxis. Specific IgE levels to whole influenza vaccines of different manufacturers and HA proteins measured using ELISA. Compared with age-matched controls, this patient showed elevated specific IgE levels against whole vaccines from egg-based manufacturing process, especially with split virion. And specific IgE levels to influenza vaccines were decreased over time. A child without egg allergy could also have an anaphylactic event to influenza vaccination, caused by elevated specific IgE levels to egg-based and split virion vaccines. In this case, cell-based and subunit vaccine could be safe from unexpected vaccine-associated anaphylaxis.

P.C5.02.19

The PDK1/TBK-1 inhibitor BX-795 is a potent suppressor of Th1 and Th2 cytokines that stimulates IL-2 secretion

P. A. Tauber*, M. R. Candia, D. Trapin, C. Köhler, A. Neunkirchner, S. Jutz, P. Steinberger, U. Smole, W. F. Pickl;*
Institute of Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria.

BX-795 is an inhibitor of 3-phosphoinositide-dependent kinase-1 (PDK1) and TANK-binding kinase-1 (TBK1), involved in T cell receptor (TCR) and innate signaling. We investigated the immunomodulatory effects of BX-795 on splenocytes of TCR/DR1 double transgenic mice and hPBMCs. We assessed viability, activation marker expression and transcription factor activity of T cells by flow cytometry and proliferation by methyl-^{3}H-thymidine uptake upon TCR ligation. Cytokine secretion was measured by multiplexing of culture supernatants, RT-qPCR and intracellular cytokine staining. Notably, BX-795 strongly inhibited secreted IFN-γ (72.9±10.0%, p<0.01) at 24 hours and also inhibited IL-4 (88.3±6.5%, p<0.05), IL-13 (75.8±8.7%, p<0.001) and IL-10 (80.8±13.1%, p<0.001) after 48 hours. After 72 hours, BX-795 also reduced secreted IL-5 and TNF-α levels.

Interestingly, Th17 cytokines were not reduced while IL-2 was strongly increased peaking at 72 hours (4.7±1.4-fold, p<0.001) in murine cells. Similar results were obtained with hPBMCs stimulated with bacterial superantigens or anti-CD3/CD28 beads. Upon TCR-specific stimulation of Jurkat T cells, BX-795 increased secreted IL-2 levels (4.0±0.38-fold, p<0.001), NFAT, NFκB and AP-1 reporter activity, and IL-2 mRNA levels while IFN-γ mRNA levels remained unchanged. In summary, BX-795 potently inhibits Th1 and Th2 while maintaining Th17 cytokines and strongly promotes IL-2 synthesis/secretion upon TCR ligation of murine and human T cells *in vitro*. Future experiments will reveal the molecular mechanisms underlying our observations and will clarify the Th2 blocking potency of BX-795 in allergen-specific asthma models *in vivo.*
Supported by the Austrian Science Fund (FWF) projects DK W1248, SFB-F4609 and the Medical University of Vienna.

P.C5.02.20
Transient expression of the major birch pollen allergen Bet v 1 in the tobacco *Nicotiana benthamiana* using *in planta* assembled TMV-based provectors

***Ö. Üzülmez**, V. Mayr, A. Tscheppe, C. Palladino, H. Breiteneder;*
Institute of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria.

Birch is the main pollen-allergen-producing tree in mid- and northern Europe. Its major allergen, Bet v 1, is considered an important inducer of tree pollen and related plant food allergies. Here, we present a platform technology for expressing plant-derived allergens in a plant system.
Agrobacterium tumefaciens, a plant pathogen, contains a Ti plasmid enabling the delivery of T-DNA into plant cells. We used two tobacco mosaic virus (TMV)-based provectors that harbor either T-DNA encoding Bet v 1 on a 3'-module or viral proteins on a 5'-module. A third provector delivered phiC31 integrase for recombining the 5'- and 3'-modules. Plants were infiltrated under vacuum while submerging leaves into a suspension of agrobacteria transformed with the corresponding plasmids. The mRNA synthesis of Bet v 1 was achieved after a successful recombination which brought the subgenomic promoter, polymerase, and allergen sequences together.
Recombinant (r) allergen, including a C-terminal hexa-histidine tag, was extracted from the leaves and isolated using Ni-NTA loaded beads. The purified rBet v 1 was able to bind both the mouse monoclonal anti-Bet v 1 antibody Bip 1 and IgE from birch pollen allergic patients' sera. After optimizing infiltration rates and incubation times of the plants, the highest expression yield of rBet v 1 was 12 mg per kg fresh leaf.
We made use of this *in planta* expression system for the first time to produce a plant-derived allergen. We will apply this system for more complex allergens in the future.
Supported by Austrian Science Fund doctoral program W1248-B30.

P.C5.02.21
CyTOF analysis of nasal tissue cell composition in allergic rhinitis patients following controlled allergen challenge

***A. Voskamp**[1], M. Gerdes[2], E. Duijster[2], K. de Ruiter[1], T. Groot Kormelink[3], S. de Jong[1], M. Yazdanbakhsh[1], E. de Jong[3], R. Gerth van Wijk[2], H. Smits[1];*
[1]LUMC, Leiden, Netherlands, [2]Erasmus MC, Rotterdam, Netherlands, [3]AMC, Amsterdam, Netherlands.

Allergic immune responses are well characterized and typically dominated by T helper 2 cells and allergen-specific IgE. Most knowledge on allergic immune responses is based on peripheral blood cells from allergic patients or from animal models. Information on tissue-specific responses is scarce, however allergen-specific immune responses are initiated locally and this information is crucial for the development of novel therapies. Nasal biopsies provide valuable information regarding the upper airway immune responses, however the number of cells obtained from these biopsies is a limiting factor. A recently developed technique, combining mass-spectrometry and cytometry (CyTOF) overcomes this limitation by measuring up to 42 phenotypic cell markers in one measurement.
In this study nasal biopsies were taken from 15 allergic rhinitis patients and 15 healthy controls, before and after nasal challenge with House Dust Mite allergen. The biopsies were digested and the cellular composition of the resulting single-cell suspensions were measured by CyTOF.
Our analysis shows clear differences in cellular composition between nasal tissue and peripheral blood. Nasal tissue contained mast cells, plasma cells and dendritic cell populations, which were absent or present in low numbers in the blood. In accordance with their atopic status, patients could be distinguished from healthy controls based on increased FcεRI expression levels on dendritic cells, monocytes and mast cells. The results will provide a comprehensive overview of the local cellular responses to allergen challenge in both allergic and healthy individuals.
This study was funded by the Longfonds.

P.C5.02.22
Role of the membrane trafficking regulator Lyst during type 1 allergic responses

***A. Westphal**, F. Niko, K. H. Lee;*
Inflammation Research Group, Institute of Clinical Chemistry, Hannover Medical School, Hannover, Germany.

Mast cells are key effector cells in human allergic disorders and inflammatory disease. The activation of mast cells via antigen-mediated triggering of FcεRI causes immediate degranulation and release of pro-inflammatory mediators in a process that involves poorly characterized vesicle transport and membrane fusion events. LYST is an evolutionary conserved regulator of lysosomal trafficking. Mutations in the LYST gene cause human Chédiak-Higashi syndrome (CHS) and the orthologous phenotype in *beige* (*Bg-J*) mice. A diagnostic feature of this severe immunodeficiency disorder is the accumulation of enlarged lysosome-related granules in a variety of cells, including mast cells. The aim of this study was to characterize role of lysosomal trafficking regulator Lyst for mast cell-mediated anaphylactic reactions.
We here report that in two different FcεRI-mediated animal disease models of passive systemic and passive cutaneous anaphylaxis, Lyst-mutant *Bg-J* mice display substantially enhanced immediate anaphylactic reactions. Subsequent mechanistic *in vitro* studies in bone marrow-derived mast cells (BMMCs) aimed at identifying the underlying mechanism surprisingly revealed normal FcεRI-mediated release of preformed mediators. However, *Bg-J* mice showed exacerbated allergic responses when injected with a mix of mediators from degranulated BMMCs, suggesting a mast cell-independent but rather tissue-dependent mechanism. Interestingly, *Lyst*-mutant BMMCs exhibited impaired *de novo* secretion of inflammatory cytokines, which correlated with impaired recruitment of inflammatory immune cells during the second phase of the anaphylactic response *in vivo*.
In summary, our data demonstrate a negative tissue-dependent regulatory role of Lyst in immediate allergic reactions and indicate a role during *de novo* secretion in mast cells.

P.C5.03 Allergy, asthma and therapy - Part 3

P.C5.03.01
A regulatory macrophage phenotype induced by IgG4: implications for allergen immunotherapy-mediated immune tolerance

***R. Bianchini**[1], F. Roth-Walter[1], A. Ohradanova-Repic[2], S. Flicker[3], M. B. Fischer[4,5], H. Stockinger[2], E. Jensen-Jarolim[1,3];*
[1]The Interuniversity Messerli Research Institute, University of Veterinary Medicine Vienna, Medical University of Vienna, Dept. of Comparative Medicine, Vienna, Austria, [2]Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria, [3]Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria, [4]Medical University Vienna, Dept. of Blood Group Serology and Transfusion Medicine, Vienna, Austria, [5]Danube University Krems, Dept. of Health Science and Biomedicine, Vienna, Austria.

Background: The presence of pro-allergic M2a macrophages is a characteristic of the immediate type of allergy and probably allergen-specific immunotherapy (AIT) is associated with the occurrence of IgG4 is considered and probably of the immunoregulatory M2b subtype. So far, no clear tolerance function is associated with IgG4. Here, we investigated whether IgG4, has implications in the conversion of the M2a into M2b-like phenotype as a new concept to explain the AIT-mediated immune tolerance. **Methods:** The *in vitro* protocol for alternative macrophage polarization was modified and M2a macrophages were incubated on plates-coated with myeloma IgG1 or IgG4, or with recombinant human anti-Phl p 5 specific-IgG1 or IgG4, to mimic the presence of immune complexes. M2a polarized cellular markers and cytokines were analyzed by flow cytometry, ELISA, and rtPCR. **Results:** Immune complexes with myeloma- and allergen-specific IgG4 pushed M2a cells to alter their phenotype and function. Down-regulation of CD163 and CD206, and a significant increase in IL-10, IL-6, TNFα and CCL1 secretion indicated a shift to an M2b-like phenotype. Fc gamma receptors (FcγRs) analyses revealed that treatment with IgG4 containing ICs resulted in almost exclusive expression of FcγRII and down-modulation of FcγRII in comparison with IgG1 treated cells (p= 0.0335) or untreated cells (p < 0.00001). **Conclusion:** Our findings indicate that the IgG4 subclass can redirect pro-allergic M2a macrophages to an M2b-like immunosuppressive phenotype. This may suggest the involvement of macrophages in tolerance induction in AIT. **Supported by FWF-project SFB F4606-B28 to EJJ.**

P.C5.03.02

Allergenic potencial of aquaculture fish due to the presence of Anisaki sp L3 proteins

M. Carballeda Sangiao[1], *A. Rojas Gomez*[1], *I. Sánchez Alonso*[2], *M. Careche Recacoechea*[3], *S. Cobacho Arcos*[2], *A. Navas Sánchez*[2], *S. Pascual del Hierro*[2], *M. González-Muñoz*[1];
[1]*Foundation for Biomedical Research Hospital La Paz, Paseo de la Castellana, 28046 Madrid, Spain, Madrid, Spain,* [2]*State Agency Superior Council of Scientific Research, CSIC, Madrid, Spain., Madrid, Spain,* [3]*State Agency Superior Council of Scientific Research, CSIC, Madrid, Spain, Madrid, Spain.*

Some feed components can be carried-over into and detected in animal products. The aim of this work was to detect *Anisakis* sp. antigens/allergens in aquaculture fish due to carry-over from fish feed. We have analyzed 4 aquaculture fish (*Scophthalmus maximus*) and 2 fish feed collected in Spanish farm. Protein extracts from fish and feed were prepared and the presence of parasite allergens was assessed by immunoblotting using anti-Ani s 4 antisera and a pool of *Anisakis* allergic patients' sera. Allergens were identified by sequencing analysis and basophil activation test was performed to assess if the allergens were functionally active. Allergens were detected in both fish and feed extracts with anti-Ani s 4 and patient' sera. In addition, both extracts were able to activate basophils. The presence of Ani s 4 was confirmed by immunoblotting inhibition and specific peptides of *Anisakis* sp. haemoglobin, enolase, Ani s 9 were identified by mass spectrophotometry. Aquaculture fish contain *Anisakis sp*. allergens and can pose an unsuspected source of parasite allergens. The presence of allergens of *Anisakis sp*. in feed suggests that these allergens are carried-over from feed.

P.C5.03.03

Regulatory T cells in cord blood of children of allergic and healthy mothers

V. Černý[1], *O. Novotná*[1], *P. Petrásková*[1], *K. Macholdová*[1], *K. Boráková*[2], *L. Prokešová*[1], *J. Hrdý*[1];
[1]*Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University, Prague, Czech Republic,* [2]*Institute for the Care of Mother and Child, Prague, Czech Republic.*

Allergic diseases represent a major issue in both clinical and experimental immunology. Despite intensive research, allergy status of the mother remains the strongest universally accepted predictor of increased risk of allergy development.
Dysregulation of balance among branches of immune response, chiefly an excess of Th2 polarization, is a key underlying cause of allergic diseases. Regulatory T cells (Treg) are crucial for maintaining the correct balance and inducing tolerance towards allergens. Functional deficiency of Treg may therefore contribute to increased risk of allergy.
We studied Treg in cord blood of children of healthy mothers (with lower risk of allergy development) and allergic mothers (with relatively higher risk) by flow cytometry, aiming to find markers which could help predict allergy development. We observed higher percentage of Treg in cord blood of high-risk children compared with lowrisk group. However, expression of several markers of Treg function (intracellular IL10 and TGF-β, MFI of FoxP3, PD1) was decreased in Treg of high-risk children. These results may be explained by an expansion of Treg population trying to compensate for inadequate regulatory capacity.
We attempted to further characterize functional ability of Treg directly (inhibition of proliferation of CFSE stained target cells) and by expression analysis of selected genes (qPCR). Using flow cytometry we also analyzed expression of Helios, a transcription factor originally considered specific for thymic-derived Treg, which may also be upregulated during T cell activation.
Work supported by AZV CR 15-26877A and Charles University research programmes Progres Q25/LF1 and SVV 260 369.

P.C5.03.04

Cytokine patterns and impaired cytotoxic activity ofNK cells in children withatopic dermatitis

E. Cetin Aktas[1], *N. Akdeniz*[1], *S. Baris*[2], *E. Karakoc-Aydiner*[3], *I. Barlan*[2], *G. Deniz*[1];
[1]*Istanbul University, Aziz Sancar Institute of Experimental Medicine, Department of Immunology, İstanbul, Turkey,* [2]*2Marmara University , Research and Training Hospital, Division of Pediatric Allergy and Immunology, İstanbul, Turkey,* [3]*Marmara University , Research and Training Hospital, Division of Pediatric Allergy and Immunology, Istanbul, Turkey.*

Atopic dermatitis (AD) is the most common chronic, and pruritic inflammatory skin disease skin disease, and primarily affects children. The role of NK cells in development of this disease is still poorly documented. The current study was undertaken to determine NK1, NK2 and NK regulatory cytokine profiles, the expression of activatory receptors as well as the cytotoxic activity of NK cells in children with AD. The study group consists of atopic (n=10), non-atopic (n=6) and healthy subjects (n=13). Patients were multisensitized and had high serum total IgE levels. Cytotoxic capacity, expression of $CD56^{bright}$ and $CD56^{dim}$ NK cell subsets, NKG2D, NKp46 and CD94 expressions, and intracellular IL-4, IL-10 & IFN-γ levels were determined by flow cytometry. The percentages of $CD3^{-}CD16^{+}CD56^{+}$and $CD56^{dim}$ NK cells were significantly diminished in atopic patients compared to healthy subjects, $CD56^{dim}CD16^{bright}$ NK cell subset was significantly increased in non-atopic children than atopic group. In contrast to diminished cytotoxic capacity, expression of $CD56^{bright}CD3^{-}IFN\text{-}\gamma^{+}$ NK cells, increased expression of activatory receptor NKp46, $CD56^{bright}CD3^{-}IL\text{-}4^{+}$ and $CD56^{bright}CD3^{-}IL\text{-}10^{+}$ NKcells in whole AD children compared to those with healthy subjects were obtained. High percentages of IL-4 and IL-10 producing NK cell subsets demonstrating an Th2 and regulatory type cytokine tendency of NK cells in AD patients. Our findings suggested impaired NK cell functions in AD patients implying high activation, accompanied by decreased cell cytotoxicity, which would contribute to the pathogenesis of disease and partially explain the tendency to the skin viral infections observed in those patients.

P.C5.03.05

Toll-like receptor 2 ligation enhances therapeutic effects of mesenchymal stem cells on murine model of asthmatic inflammation

H. Yu, ***T. Chen****, B. Chiang;*
Graduate Institute of Clinical Medicine, Taipei City, Taiwan.

Introduction: Under current therapeutic strategies, the cure to asthma remains elusive; thus- novel approaches for treating asthma are desperately needed. Despite that mesenchymal stem cells (MSCs) have recently been established as potential candidates by virtue of their immunomodulatory properties, the underlying heterogeneity of MSCs diminishes their therapeutic efficacy.
Materials and Methods: Here we evaluated a toll-like receptor (TLR) 2 ligation protocol of MSCs to augment their therapeutic benefits on asthma.
Results: We surmise that a TLR2 ligand, Pam_3CSK_4, enhanced the therapeutic effects of MSCs on asthmatic inflammation in mice at low concentration (1 μg/mL) for a longer induction period (96 h) in a post-treatment manner. We further validated this regimen by demonstrating that Pam_3CSK_4 activated STAT3 in BM-MSCs through IL-6, which was likely stimulated with NF-κB signaling. NO, the key suppressive molecule of Pam_3CSK_4-primed BM-MSCs, was later highly increased through upregulation of iNOS, which was in the downstream of STAT3 phosphorylation. The intensified suppressive functions of BM-MSCs were then executed by inducing $CD4^{+}CD25^{+}Foxp3^{+}$ regulatory T cells in a post-treatment manner.
Conclusion: The results demonstrated that TLR2 ligand, Pam_3CSK_4, could modulate the function of BM-MSCs and alleviate airway inflammation. The consistent therapeutic outcomes and the valid suppressive mechanisms in the study might shed light on eliminating the proinflammation-prone uncertainties of MSCs, and enhancing the future feasibility of obtaining long-term effects with this regimen.

P.C5.03.06

Are eosinophils major contributors to oxidative stress during asthma exacerbations?

L. E. S. de Groot[1], *Y. S. Sabogal Piñeros*[1], *S. M. Bal*[1], *M. A. van de Pol*[1], *W. Kulik*[1], *T. A. van der Veen*[2], *B. N. Melgert*[2], *F. O. Martinez*[3], *J. Hamann*[1], *R. Lutter*[1];
[1]*Academic Medical Center, Amsterdam, Netherlands,* [2]*University of Groningen, Groningen, Netherlands,* [3]*University of Surrey, Guildford, United Kingdom.*

Introduction: Asthma exacerbations are predominantly triggered by respiratory viral infections and characterized by eosinophilic airway inflammation and increased oxidative stress. Eosinophils can produce reactive oxygen species (ROS) and a link between eosinophils and oxidative stress during exacerbations is thus likely. Attenuation of eosinophils using anti-IL-5 (Mepolizumab) in severe asthmatics significantly reduces exacerbation rates and corticosteroid dependency. Yet, the impact of reduced eosinophils on ROS production has not been investigated so far. Therefore, we aimed to study the contribution of eosinophils to oxidative stress during virus-induced asthma exacerbations.
Methods: Mild asthmatics received one high dose of Mepolizumab or placebo and after two weeks were subjected to rhinovirus 16 (RV16) infection. Exhaled breath condensate was collected before and after RV16 and levels of malondialdehyde (MDA), asymmetric dimethylarginine, nitrotyrosine, bromotyrosine, chlorotyrosine and dityrosine were measured using ultra-performance liquid chromatography-tandem mass spectrometry.
Results: Mepolizumab effectively attenuated eosinophil numbers in the circulation and locally in the airways. Oxidative stress levels before RV16 challenge were comparable between the groups. MDA near-significantly and nitrotyrosine significantly increased after virus exposure in the placebo group, but not in the Mepolizumab group. When stratified for patients with high and low eosinophil counts, MDA levels significantly increased in the high eosinophilic placebo group only.
Conclusion: RV16 infection in mild asthmatics is accompanied by enhanced oxidative stress levels, which can be ameliorated by Mepolizumab. Our findings suggest that eosinophils drive oxidative stress during virus-induced asthma exacerbations.
This work is supported by the Lung Foundation Netherlands (4.1.15.002 and 3.2.10.069) and GSK (CRT 114696).

P.C5.03.07

Amelioration of itch and reduced mast cell degranulation by single-stranded oligonucleotides

A. Dondalska[1], *E. Rönnberg*[2], *H. Ma*[3], *S. Pålsson*[1], *E. Magnusdottir*[3], *L. Adam*[1], *G. Nilsson*[2], *M. Lagerström*[3], *A. Spetz*[1];
[1]*Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm, Sweden,* [2]*Immunology and Allergy Unit, Department of Medicine, Karolinska Institute, Solna, Sweden,* [3]*Department of Neuroscience, Uppsala University, Uppsala, Sweden.*

Introduction: Itch is a large problem in numerous skin disorders. The Mas-related G protein-coupled receptor X2 (MRGPRX2) has been shown to modulate itch by inducing non-IgE-mediated mast cell degranulation and the release of endogenous inducers of pruritus such as histamine. Various basic cationic substances, including inflammatory peptides and certain drugs, can trigger MRGPRX2 and thereby induce pseudo-allergic reactions characterized by histamine release and inflammation. Here, we investigated the capacity of an immunomodulatory single-stranded oligonucleotide (ssON) to inhibit itch and mast cell degranulation. Materials and Methods: To determine the effect of ssON on itch, we performed behavioral studies in established mouse models and collected skin biopsies. We examined the effect of ssON on MRGPRX2 activation *in vitro* by measuring degranulation (flow cytometry, ELISA) in a human mast cell line and calcium influx in MRGPRX2-transfected cells. Results: We observed that intradermal injection of ssON in mice was able to inhibit itch induced by the basic secretagogue C48/80 in a dose-dependent manner. Evaluation of histological staining at the injection site revealed that ssON appeared to inhibit mast cell degranulation in murine skin treated with C48/80. SsON also demonstrated the capacity to inhibit MRGPRX2 activation *in vitro* by blocking mast cell degranulation and calcium influx in MRGPRX2-transfected cells induced by certain basic secretagogues in a dose-dependent manner. Conclusions: SsON can inhibit IgE-independent mast cell degranulation and itch. Since there is a need for small molecules to block MRGPRX2-mediated activation of mast cells, ssON could be utilized to ameliorate itch in certain pathological settings

P.C5.03.08

The non-obese diabetic mouse as a novel model for severe allergic asthma: the respective roles for T_H2 and IL-17-producing cells

A. Foray[1,2,3], *C. Marquet*[1,2], *C. Pecquet*[1,2], *C. Dietrich*[1,3], *F. Machavoine*[1,3], *M. Dy*[1,2,3], *J. Bach*[1,2,3], *L. Chatenoud*[1,2,3], *M. Leite de Moraes*[1,2,3];
[1]*Faculté de Médecine Paris Descartes, Université Paris Descartes, Paris, France,* [2]*Institut National de la Santé et de la Recherche Médicale, Unité 1151, Laboratory of Immunoregulation and Immunopathology, Institut Necker-Enfants Malades, Paris, France,* [3]*Centre National de la Recherche Scientifique, UMR 8253, Laboratory of Immunoregulation and Immunopathology, Institut Necker-Enfants Malades, Paris, France.*

Paradoxically, the Non-Obese Diabetic (NOD) mouse, a prototypic model of T_H1-mediated autoimmune diabetes, is highly prone to develop allergic reactions. Here, in a classical house-dust mite (HDM)-induced asthma model, we compared NOD and BALB/c mice responses and dissected the pattern of cytokine-producing cell subsets *in situ*.
NOD mice exhibited exacerbated airway hyper-reactivity as assessed, using the FlexiVent device, by the resistance after methacholine challenge (10±1.9 vs. 4±0.8 $cmH_2O.s.mL^{-1}$ at 25mg MCh, in NOD and BALB/c respectively, *P*<0.01). Allergic airway inflammation was enhanced, as reflected by increased eosinophil counts in the bronchoalveolar lavage fluids of NOD mice ($5.1x10^5$ cells±0.6 vs. $0.7x10^5$±0.2, *P*<0.01), as were circulating anti-HDM IgG1 levels (0.76±0.15 vs. 0.26±0.01 OD, *P*<0.01). Interestingly, the pattern of cytokine-producing cells was clearly distinct in the lungs of asthmatic NOD mice as compared to BALB/c. First, the proportions of IL-4, IL-13 and IL-5 producing $CD4^+$ T cells were increased (13.2%±3.1 vs. 6.7%±0.6, 9.1%±0.5 vs. 2.7%±0.2, and 5.9%±0.6 vs. 2.0%±0.3 respectively, all *P*<0.01). Secondly, CD4+ T cells were the major IL-5 producers in NOD mice (60.8±4.0% $TCR\beta^+CD4^+$ among $IL\text{-}5^+$ cells) while in BALB/c the source of IL-5 were type 2 ILCs (60.6±6.7% Lin^-CD127^+). Third, an exacerbated production of IL-17 was found of NOD mice which sources included conventional $CD4^+$ T cells (3.1%±0.3 vs. 1.1%±0.2, *P*<0.01), iNKT17 cells (11.2%±1.5 vs. 1.6%±0.1, *P*<0.01) and IL-17-producing $V\gamma1^+$ T cells (2.8%±0.4 vs. 0.9%±0.4, *P*<0.05). Our results demonstrate that the NOD strain develops a unique form of severe allergic-induced asthma associated with exacerbated TH2 and IL-17-biaised immune responses.

P.C5.03.09

Efficacy of montelukast in the treatment program of persistent atopic asthma

I. Garmish, *O. Shtepa;*
SE "Dnipripetrovsk medical academy of Health Ministry of Ukraine", Dnipro, Ukraine.

The aim of our study was to evaluate the effectiveness of montelukast in the treatment program of persistent atopic asthma in women and to estimate the diagnostic value of general serum IgE and forced expiratory volume on first second (FEV_1) during the decreasing level of bronchial obstruction. Materials and Methods. We observe 95 patients (pts) with persistent atopic asthma. They were divided into groups: main group - 60 pts who received montelukast (10 mg, orally), as add-on therapy to inhaled corticosteroids (ICS) and bronchodilators, group of compared - 35 pts who received only ICS and bronchodilators. IgE antibodies were assayed with electrochemiluminescence immunoassay analyzer in addition to general examination and spirometry. Results. This study indicated that main group IgE levels were 306,5+/-17,98 IU/ml, while the group of compared - 301,1+/-26,16 IU/ml. Following the montelukast therapy there was a statistically difference in general IgE levels (p□0.001) in main group (IgE 128,6+/-8,54 IU/ml) and wasn□t significantly reduced (p□0.05) in group of compared (IgE 295,6+/-8,54 IU/ml). The indicators of the FEV_1 at main and comparison groups after treatment significantly increased (p□0.05), also there was statistically difference (p□0.05) between main group (FEV_1 - 82,2+/-0,18 %) and group of compared (FEV_1 - 87,3+/-0,32 %). The data indicated a correlation between serum IgE levels and FEV_1 (R=0,712, p□0.05). Conclusions. Montelukast significantly better increase FEV_1 and decrease general IgE level, improving clinical, functional manifestations of persistent atopic asthma in women and increasing anti-inflammatory effects.

P.C5.03.10

Structure and biochemical characterization of wheat β-amylase, a clinically relevant food allergen

G. Hofer[1], *S. Wieser*[2], *M. Bogdos*[1], *P. Gattinger*[2], *R. Valenta*[2], *W. Keller*[1];
[1]*Institute of Molecular Biosciences, University of Graz, Graz, Austria,* [2]*Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria.*

Introduction: Wheat is one of the most important staple foods worldwide and has been recognized as a potent allergen source. However not all known potential allergens of wheat have been characterized so far. The wheat β-amylase has been found to bind IgE of wheat allergic patient, but its structure and allergenic activity have not been studied.
Methods: Wheat β-amylase was expressed in native and non-native (aggregated) forms in *E. coli* and biochemically characterized by size exclusion chromatography, circular dichroism spectroscopy, differential scanning fluorimetry and activity assays. Finally, the enzyme was crystallized and the structure solved to 2 Å.
Results: The crystal structure of wheat β-amylase shows the same $(\beta/\alpha)_8$-barrel architecture found for other plant and bacterial β-amylases. The enzyme shows a tolerance for low pH, showing maximum activity at pH 5 and retaining over 80% of its maximum activity even at pH 4. However, more than 50% activity is lost at pH 8. The protein is most thermally stable in slightly acidic conditions. A maximum in melting temperature is seen at pH 5.5 with 59°C, with a tail off to lower values as pH increases and a sharp drop in stability at pH 4.
Conclusion: The recombinant expression of natively folded wheat β-amylase was successful and provides a useful tool for screening since the IgE recognition of allergic patients shows a clear difference between natively and non-natively folded recombinant β-amylase.

P.C5.03.11

Analysis of nutrient composition of breast milk with the allergic severity in infantile atopic dermatitis

Y. Huang[1], *L. Wang*[2], *B. Lin*[3], *B. Chiang*[2];
[1]*Department of Pediatrics Taipei Medical University-Wan Fang Hospital, Taipei City, Taiwan,* [2]*Department of Pediatrics National Taiwan University Hospital, Taipei City, Taiwan,* [3]*Department of Biochemical Science and Technology, College of Life Science, National Taiwan University, Taipei City, Taiwan.*

Introduction: Atoptic dermatitis (AD) is a chronic allergic skin disease with a typical symptom of pruritic rash. We aim to determine the differences of nutrient components in the breast milk, maternal diet pattern analysis during lactation, between atopic dermatitis and healthy infants. Methods: We conducted a prospective study to determine the differences of components(oligosaccharides, microbiota, Vitamin A, Vitamin D and fatty acid), in the breast milk, between infantile AD and healthy infants. We collected breast milk at 2 and 6 month postpartum and the mothers are interviewed by the dietician for the diet questionnaireat (diet pattern analysis) at the same time.Results: We enrolled 87 infants in our prospective study, of which 52 infants with infantile atopic dermatitis (study group) and 35 of them was healthy infants (control group). Infants with infantile atopic dermatitis might have a tendency of having parental history of atopic dermatitis (N=35, 81.4%, P value=0.27) than that of healty infants (N=16, 69.6%,P value=0.27). The fatty acids analysis of breast milk at 2-month post-partum showed that no significant difference between two groups. However, Vitamin D level in the breast milk for healthy infants was higher then those for infants with atopic dermatitis (P value < 0.01).Conclusion. It is suggested that dietary factors might play a critical role in immune modulation and disease severity of allergic diseases. We demonstrated that lower vitamin D level was noted in the breast milk of mother having baby with infantile atopic dermatitis.

P.C5.03.12
Characterization of urticaria in children

P. H. Huang[1,2], *J. H. Lee*[2], *B. L. Chiang*[2];
[1]*Department of Pediatrics, Cardinal Tien Hospital, New Taipei City, Taiwan,* [2]*Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan.*

Introduction: The etiologies of acute urticaria vary, with the leading cause is infection in pediatric group. In contrast, infection only accounts for a minority of cause in the adult group. A previous study indicated that atopic diseases are related to acute urticaria, but not chronic urticaria. In addition, the percentage of acute versus chronic urticaria are different in children and adults. This study is to evaluate the age distribution of urticaria types, etiologies, and relationship with atopy in children.
Material and methods: Patients' data are obtained retrospectively by chart review. Types of urticaria are classified according to 2018 revision of EAACI/GA2LEN/EDF/WAO Guidelines for Definition, Classification, Diagnosis and Management of Urticaria. Identification of etiologies are from notes and questionnaires to the family if necessary. The included patients are divided into 4 age groups (0-<3, 3-5, 6-11, 12-18) to compare the differences among age groups.
Results: The results showed the ratio of acute versus chronic urticaria is 4:1. The overall age statistics revealed a male predominance and the female/male ratio is 0.76. Of all the identifiable etiologies, infection is the leading cause of acute urticaria (33.5%), whereas there is only 2.4% patients with chronic urticaria caused by infection. Allergic diseases were found in over one third patients with acute urticaria while only 11% in that with chronic urticaria.
Conclusion: Our findings suggested there was much difference in etiologies and relationship with atopy between acute and chronic urticaria in overall age groups. Further analysis addressing the different age groups will be further reported.

P.C5.03.13
The transcription factor EPAS1 links DOCK8 deficiency to atopic skin inflammation via IL-31 induction

K. Kunimura[1], *K. Yamamura*[1], *Y. Fukui*[1,2];
[1]*Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan,* [2]*Research Center for Advanced Immunology, Kyushu University, Fukuoka, Japan.*

Introduction: Mutations of DOCK8 in humans cause a combined immunodeficiency characterized by atopic dermatitis. However, the molecular link between DOCK8 deficiency and atopic skin inflammation is unknown. Materials and Methods: The skin disease development was compared between Dock8$^{-/-}$ and Dock8$^{+/-}$ mice expressing TCR transgene. After stimulation with the cognate antigen, CD4$^+$ T cells were used for cytokine production assay, microarray analysis and ChIP assay. IL-31 promoter activation, EMSA and immunoprecipitation were performed by standard techniques. Nuclear translocation of EPAS1 was examined in mouse embryonic fibroblasts generated from wild-type and Dock8$^{-/-}$ mice.
Results: We found that unlike Dock8$^{+/-}$ mice, Dock8$^{-/-}$ mice spontaneously developed severe atopic skin inflammation, when crossed with transgenic mice expressing TCR with a particular antigen specificity. Upon stimulation, CD4$^+$ T cells from Dock8$^{-/-}$ mice produced large amounts of IL-31, a major pruritogen associated with atopic dermatitis. This IL-31 induction critically depended on the transcription factor EPAS1, and its conditional deletion in CD4$^+$ T cells abrogated skin disease development in Dock8$^{-/-}$ mice. Although EPAS1 is known to form a complex with aryl hydrocarbon receptor nuclear translocator (ARNT) and control hypoxic responses, EPAS1-mediated Il31 promoter activation was independent of ARNT, but in collaboration with SP1. In addition, we found that DOCK8 acted as an adaptor and negatively regulated nuclear translocation of EPAS1. Conclusions: EPAS1 links DOCK8 deficiency to atopic skin inflammation via IL31 induction in CD4$^+$ T cells.

P.C5.03.14
The role of CD133 in the development and immune modulation of SSEA-1$^+$ lung stem/ progenitor cells

C. Liao[1], *C. Chiu*[1], *B. Chiang*[1,2];
[1]*Graduate Institute of Immunology, College of Medicine, National Taiwan University, Taipei, Taiwan,* [2]*Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan.*

Introduction: In the previous studies, we had identified a subset of lung stem/ progenitor cells from neonatal mice lungs, SSEA-1$^+$ pulmonary stem/ progenitor cells (SSEA-1$^+$PSCs). SSEA-1$^+$PSCs are able to self-renew and differentiate into airway ciliated and alveolar cells. Interestingly, SSEA-1$^+$PSCs but not SSEA-1$^-$ cells are capable to suppress allergic airway inflammation in OVA-induced allergic asthma mouse model. However, we found that SSEA-1$^+$PSCs are further divided into two cell populations by CD133 expressing or not. In the present studies, we analyzed the stem-ness and functions of CD133$^+$SSEA-1$^+$PSCs and CD133$^-$SSEA-1$^+$PSCs. **Materials and Methods:** CD133$^+$SSEA-1$^+$PSCs and CD133$^-$SSEA-1$^+$PSCs were isolated from neonatal mice lungs. Cells were analyzed by qPCR, cell differentiation assays and 3D culture. OVA-induced allergic airway inflammation were used to analyze the immuno-regulatory function of the cell populations. **Results:** In comparison with the gene expression profile, CD133$^+$SSEA-1$^+$PSCs are expressed higher levels of airway-related genes than CD133$^-$SSEA-1$^+$PSCs. Although both the cell populations could differentiate into alveolar cells, only CD133$^+$SSEA-1$^+$PSCs are able to differentiate into airway ciliated cell. To evaluate the stem/ progenitor properties, cells are subjected to 3D culture. We found that both CD133$^+$SSEA-1$^+$PSCs and CD133$^-$SSEA-1$^+$PSCs generated organoids during 3D culture, suggesting the stem-ness of both cell populations. To determine the immuno-regulatory ability of CD133$^+$SSEA-1$^+$PSCs and CD133$^-$SSEA-1$^+$PSCs in allergic airway inflammation, cells are intravenously transferred into murine model of asthma. Results showed that the allergic airway inflammation are suppressed by both cell populations. **Conclusions:** We demonstrated that neonatal mice lung-derived CD133$^+$SSEA-1$^+$ and CD133$^-$SSEA-1$^+$ cell populations exhibited the stem cell activities and immuno-regulatory functions.

P.C5.03.15
Effect of chronic stress in a mouse model of contact hypersensitivity

A. MacKerracher[1], *A. Sommershof*[1], *M. Groettrup*[1,2];
[1]*University of Konstanz, Konstanz, Germany,* [2]*Biotechnology Institute Thurgau, Kreuzlingen, Switzerland.*

Chronic stress is known to have a suppressive effect on the immune system via the secretion of glucocorticoids mediated by the hypothalamus-pituitary-adrenal (HPA) axis. Interestingly, chronic stress is known to increase the susceptibility and disease progression of inflammatory skin diseases in humans. We studied inflammatory skin reactions in a well-established mouse model of contact dermatitis termed contact hypersensitivity (CHS) using the established contact allergens DNTB (2,4-dinitrothiocyanobenzene) and DNFB (2,4-dinitrofluorobenzene). DNTB is a weak contact allergen that does not trigger allergic reactions in non-allergic individuals, but instead has been shown to induce tolerance to the strong contact allergen DNFB. Tolerance induction in this model is facilitated by Langerhans cells (LC), which migrate from the epidermis to draining lymph nodes (dLNs), where they activate regulatory T cells and lead to the inactivation of specific cytotoxic CD8+ T lymphocytes. We have shown that tolerance induction in response to the weak contact sensitizer is strongly reduced in stressed mice. Furthermore we were able to prove that the reduced tolerance induction is accompanied by significantly impaired migration of LCs from the epidermis to dLNs following application of DNTB. We could further show that impaired migration of LCs in stressed mice is due to a reduced activation of LCs in the sensitized skin. Collectively our results suggest that chronic stress alters the reactivity towards contact allergens leading to a higher susceptibility to establish CHS reactions even towards weak contact allergens.

P.C5.03.16
Immunological effects of omalizumab in a group of long-term treated asthma patients

L. Maggi[1], *B. Rossettini*[1], *G. Montaini*[1], *A. Matucci*[2], *A. Vultaggio*[2], *A. Mazzoni*[1], *E. Maggi*[1], *F. Liotta*[1], *F. Annunziato*[1], *L. Cosmi*[1];
[1]*University of Florence, Florence, Italy,* [2]*Azienda Ospedaliero-Universitaria Careggi, Florence, Italy.*

Background: The anti-IgE monoclonal antibody, omalizumab, has proven to be effective in the treatment of allergic severe uncontrolled asthma. The aim of the study was to evaluate the immunological effects of omalizumab, in a group of severe allergic asthma patients, treated since at least three years
Methods: Immune cell subsets have been evaluated in a cohort of 15 allergic asthmatic patients treated with omalizumab, and the data have been compared with 12 allergic asthma patients treated with standard therapy.
Results: Omalizumab treated asthmatic patients showed lower frequencies of circulating plasmacytoid dendritic cells, and lower CD154 expression on CD4 T helper cells respect to the control group. Moreover, basophils and DCs from omalizumab treated patients had lower levels of IgE on their surface respect to the control group, and this was the consequence of the reduction of FcεRI expression, but also some FcεRI free of IgE were detected. In a longitudinal evaluation of two patients that started omalizumab treatment, the presence of FcεRI free of IgE was evident just after the first administration of the drug. Finally we performed in vitro experiments on basophils obtained from healthy donors, that show that omalizumab is able to detach IgE from their receptors.
Conclusions: Collectively these data indicate that long-term omalizumab treatment dampen type 2 inflammation acting on different cell types that play a pivotal role in the pathogenesis of allergic asthma, Moreover a possible novel activity of omalizumab, the ability to detach IgE from their receptors, is suggested.

P.C5.03.17

Targeting the IL-7R alpha as a potential therapeutic approach for allergic asthma

H. L. Mai[1,2], *E. Nguyen*[1,2], *G. Bouchaud*[3], *K. Henrio*[4], *M. Cheminant*[4], *S. Dehmani*[1,2], *A. Magnan*[4], *S. Brouard*[1,2];
[1]*Centre de Recherche en Transplantation et Immunologie UMR 1064, INSERM, Université de Nantes, Nantes, France,* [2]*Institut de Transplantation Urologie Néphrologie, CHU Nantes, Nantes, France,* [3]*INRA BIA UR 1268, Nantes, France,* [4]*INSERM UMR 1087, Institut du Thorax, Nantes, France.*

Introduction: Asthma remains an important cause of morbidity and mortality. We herein provide for the first time a preclinical proof-of-concept for a novel therapeutic approach for allergic asthma using an anti-IL-7Rα mAb, which blocks both IL-7R and TSLPR. Methods: We used a murine asthma model in which mice received 4 weekly percutaneous sensitizations followed by 2 weekly intranasal (IN) challenges with total house dust mite (HDM) extracts as allergen. This model corresponds to a mixed asthma phenotype in which the bronchoalveolar inflammation comprises both neutrophils and eosinophils. Asthmatic mice were then treated with an anti-IL-7Rα mAb or an isotype control every other day during the 2 weeks of IN challenges. Results: Anti-IL-7Rα mAb blocks STAT5 phosphorylation in mouse CD4+ T cells induced by either IL-7 or TSLP. Compared to control animals, anti-IL-7Rα-treated mice showed significantly lower airway resistance in response to methacholine as measured by flexiVent, associated with an improvement in lung histology. Anti-IL-7Rα treatment significantly decreased the mRNA expression of Th2 cytokines (IL-4, IL-5, and IL-13) and chemokines (CCL5/RANTES) in lung tissue, decreased the secretion Th2 cytokines (IL-4, IL-5, and IL-13) and chemokines (CXCL1 and CCL11/eotaxin) in bronchoalveolar lavage fluid (BALF) as measured by luminex, and decreased serum HDM-specific IgE as measured by ELISA. Leukocyte phenotyping by flow cytometry revealed a reduction of eosinophils, total lymphocytes, T cells, and especially ILC2 in lung and BALF of anti-IL-7Rα-treated mice. Conclusion: Targeting the IL-7Rα by a mAb, through its broad mechanisms of action, presents as a potential therapeutic approach for asthma.

P.C5.03.18

Whole-proteome profiling of primary human mast cells reveals evolutionary conservation of cell-type specific pathways

T. Plum[1], *T. Feyerabend*[1], *M. Rettel*[2], *J. Krijgsveld*[1], *H. Rodewald*[1];
[1]*German Cancer Research Center, Heidelberg, Germany,* [2]*European Molecular Biology Laboratory, Heidelberg, Germany.*

Mast cells are bone marrow-derived immune cells that are present in tissues lining the environment. In these tissues mast cells sense environmental allergens, and upon recognition, become activated to release granules and generate inflammatory mediators. Mast cell-derived products confer protection against snake venoms and some parasite infections. However, aberrant activation of mast cells contributes to pathologies such as allergy, asthma and adverse drug reactions. To better understand the biology of mast cells and to identify novel functions, we assessed the proteome of primary human mast cells isolated from two different anatomical compartments by quantitative mass spectrometry. Using this approach, we identified a mast cell-specific proteomic signature consisting of prototypic mast cell markers such as KIT and FcεRI as well as proteins like VWA5A and FAM129B that were highly enriched in mast cells but for which no function has been reported yet. Protein expression of the most enriched mast cell-specific cell surface proteins was verified by flow cytometry, demonstrating marked differences between connective-tissue-type and mucosal-type mast cells in humans. We also compared proteome signatures of human and mouse mast cells which revealed striking conservation across species, in particular in pathways governing granule biosynthesis and secretion, as well as proteoglycan- and neurotransmitter metabolism, suggesting evolutionary maintenance of mast cell functions. This mast cell protein expression atlas shall aid in uncovering novel functions for this enigmatic cell type.

P.C5.03.19

Dupilumab in real life practice: patient's atopic profile, efficacy on atopic dermatitis and change in specific IgE

M. Tauber[1,2], *P. Apoil*[3], *C. Richet*[1], *G. De Bonnecaze*[4], *E. Mouchon*[4], *M. Cassagne*[5], *M. Marguery*[1], *S. Hegazy*[1], *M. Konstantinou*[1], *M. Severino*[1], *C. Uthurriague*[1], *F. Giordano-Labadie*[1], *A. Didier*[6], *C. Paul*[1,2];
[1]*Toulouse University, Dermatology and Allergology Department, Larrey Hospital, Toulouse, France,* [2]*INSERM-Paul Sabatier Toulouse University, U1056 UDEAR, Purpan Hospital, Toulouse, France,* [3]*Toulouse University, Immunology Department, Rangueil hospital, Toulouse, France,* [4]*Toulouse University, Ear Nose and Throat Department, Larrey Hospital, Toulouse, France,* [5]*Toulouse University, Ophtalmology Department, Pierre Paul Riquet Hospital, Toulouse, France,* [6]*Toulouse University, Pneumology and Allergology Department, Larrey Hospital, Toulouse, France.*

Introduction. Dupilumab is an antagonist of the Interleukin-4/13 receptors and was recently approved in Europe for use in adults with moderate-to-severe atopic dermatitis (AD). Our objective was to assess the impact of dupilumab therapy on both clinical (cutaneous, pulmonary, nasal and ocular symptoms) and biological (total and specific IgE, sIgE) parameters in real-life practice. Methods. Dupilumab was administered as labelled in the context of a Temporary Use Authorisation. Patients were evaluated initially (week 0, W0) and 16 weeks (W16) after the first injection. Follow-up parameters were: SCORing AD (SCORAD), visual analogue scale (VAS) for sleep and pruritus, Dermatology Life Quality Index (DLQI), Asthma Control Test (ACT), fraction of exhaled nitric oxide (FeNO), expiratory flow-volume curves, ophthalmologic and nasal symptoms, dosages of total and sIgE, using both single-tests and ImmunoCAP™ ISAC 112 biochip. Results. Nineteen patients were included (median age: 38 years). The median SCORAD was 49 at W0. SCORAD, DLQI, pruritus and sleep VAS decreased significantly at W16. There was no difference for ACT or the flow-volume curves but a significant decrease was observed for the FeNO. Eight patients presented either a de novo conjunctivitis (n=3) or a worsening of their ophthalmologic symptoms at W16. Four patients improved their rhinitis and one experienced worsening. Total IgE, sIgE against Malassezia or Staphyloccocus toxins, and ISAC sIgE (total of 517 values) decreased significantly at W16. However, there was no correlation with the SCORAD improvement. Conclusion. Dupilumab improves AD condition in real-life practice and modifies the sIgE sensitisation profile of the patients.

P.C5.03.20

Plasma cytokine profiles during peanut oral immunotherapy in severely peanut allergic adolescents.

M. van der Heiden[1], *C. Carvalho-Queiroz*[1], *C. Nilsson*[2,3], *A. Nopp*[3], *E. Sverremark-Ekström*[1];
[1]*Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden,* [2]*Sachs' Children and Youth Hospital, Södersjukhuset, Stockholm, Sweden,* [3]*Department of Clinical Science and Education, Södersjukhuset, Karolinska Institutet, Stockholm, Sweden.*

Peanut allergy is a major cause of anaphylaxis, hence effective treatment strategies for peanut allergy are warranted. Our previous work has shown that individualized omalizumab treatment (OT) allows a safe initiation and rapid up-dosing of peanut oral immunotherapy (pOIT) in severe peanut allergic adolescents.
We aimed to longitudinally follow the cytokine profiles in plasma samples of severely peanut allergic adolescents (n=19) undergoing combined OT and pOIT and subsequently compare these profiles between treatment failures (TF) (n=6) and successes (TS) (n=13).
Concentrations of plasma cytokines (IL2, IL4, IL5, IL9, IL10, IL12p70, IL13, IL21, GM-CSF, IFNg, TNFa) were determined by multiplex bead-based immunoassays and ELISA at 4 different time-points; before starting OT (baseline), at the peanut challenge after OT prior to starting pOIT (challenge), at maintenence dose pOIT prior to OT reduction (stepdown), and at the final peanut protein challenge (final visit).
Cytokine levels were relatively low and stable between the time-points. We noted an increase of IL-5 and IL-9 levels at the maintenence dose pOIT prior to OT reduction (stepdown time-point) in most individuals. Further, we observed higher concentrations of GM-CSF at the challenge time-point in the TF group.
These data indicate that most individuals have an ongoing Th2/Th9-type of response during pOIT. The higher GM-CSF levels at the challenge time-point in the TF group suggest a more active basophil compartment in these individuals. Future research will focus on chemokine responses as well as B and T cells underlying the treatment.

P.C5.03.21

Human immunoglobulin: an effective treatment for severe atopic dermatitis

P. E. Walo Delgado[1], *P. Lapuente Suanzes*[1], *A. Roncancio Clavijo*[1], *M. A. Ballester Martinez*[2], *A. De Andres Martin*[1];
[1]*Servicio de Inmunología, Madrid, Spain,* [2]*Servicio de Dermatología, Madrid, Spain.*

Introduction. Atopic dermatitis is the most prevalent inflammatory skin disease. Currently, there is a wide range of treatments that include phototherapy, topical treatment, cyclosporine and biological therapy with monoclonal antibodies. In severe cases, patients undergo prolonged therapy and high doses of drugs, which leads to the appearance of more adverse effects and comorbidity, such as increased risk of infections. **Materials and method.** We included 3 patients (42-year-old men, 58-year-old women, and 27-year-old women) with severe atopic dermatitis who had failed conventional treatment (including phototherapy, prednisone, cyclosporine, and Omalizumab). We have treated them with human immunoglobulin (IVIG), at doses of 0.8 mg / kg every 3-4 weeks, after signing informed consent and approval by the pharmacy committee of our hospital. **Results** All 3 patients had an important improvement of their symptom, with decreased pruritus and eczema. Two patients with asthma have presented improvement of respiratory symptoms. No adverse effects related to the treatment have been reported, except post-infusion headache. **Conclusion.** IVIG is an effective and safe alternative to treat severe atopic dermatitis due to its high capacity of immunomodulation. However, due to its high cost and limited production, it should be used in cases of refractory patients or when conventional treatment is contraindicated due to comorbidities.

P.C5.03.22

Structural characterization of different profilin allergens towards IgE-epitope prediction

***J. Wortmann**, J. Schöpp, A. M. Reisenbichler, G. Hofer, W. Keller;*
Institute of Molecular Biosciences, University of Graz, Graz, Austria.

Members of the profilin family are important panallergens causing respiratory allergy and oral allergy syndrome. Because of the high structural conservation within this family, profilin allergens serve as important targets for IgE-binding and epitope prediction studies. The aim of this project is the structure determination and biophysical characterization of five profilin allergens. The folded, recombinantly produced proteins will also be used for experimental determination of cross-reactivity between important respiratory as well as food allergens. The profilin family members included in this research project are the food allergens Cuc m 2 from melon (Cucumis melo) and Cit s 2 from orange (Citrus sinensis), the pollen allergens from olive tree Ole e 2 (Olea europaea), and timothy grass Phl p 12 (Phleum pratense) as well as the allergen Tyr p 36 from the storage mite Tyrophagus putrescentiae.
So far, all five profilin allergens could be recombinantly expressed and purified. Biophysical characterization including Circular Dichroism, Differential Scanning Fluorimetry and Size Exclusion Chromatography revealed the structural integrity of the allergens. Obtaining immunological data regarding cross-reactivity will enable more accurate IgE-epitope predictions using a structure based IgE-epitope mapping approach (Dall'Antonia et al.).

P.C5.04 Allergy, asthma and therapy - Part 4

P.C5.04.01

Activated intestinal epithelial cells conditioned with 2'-Fucosyllactose and CpG-ODN might instruct moDC to drive Th1 differentiation

***V. Ayechu-Muruzabal**[1], A. Kostadinova[1], S. Overbeek[2], B. Stahl[2], J. Garssen[1], B. van't Land[2], L. Willemsen[1];*
[1]Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht, Netherlands, [2]Nutricia Research, Utrecht, Netherlands.

The immunomodulatory capacities of 2'-Fucosyllactose (2'FL) were compared to short chain galacto- and long chain fructo-oligosaccharides 9:1 (GF), which previously showed to promote Th1 and regulatory type immune polarization in the presence of CpG in an *in vitro* co-culture model. Additionally, the ability of 2'FL-exposed intestinal epithelial cells (IEC) to instruct immature monocyte derived dendritic cell (moDC) function was evaluated.
IEC co-cultured with anti-CD3/CD28 activated peripheral blood mononuclear cells (PBMC), was apically exposed to 2'FL, GF or 2'FL/GF mixture (0.25, 0.5, or 1.0 w/v%) either or not combined with CpG (0.5 uM). IEC were washed and co-cultured with moDC. moDC were then used in an allogeneic assay where their capacity to induce naïve CD4+ T-cell differentiation was evaluated.
In presence of CpG, GF as well as 2'FL and 2'FL/GF enhanced IFN-gamma and IL-10 secretion of activated PBMC co-cultured with IEC compared to CpG alone ($p<0.05$), while IL-13 and IL-5 remained low. IEC-derived galectin-3, TGF-beta1 (both $p<0.001$), galectin-9 and galectin-4 (both $p<0.05$) of CpG-exposed cells was further increased by GF, 2'FL and/or 2'FL/GF compared to CpG alone. Only moDC co-cultured with activated IEC conditioned with 2'FL and CpG increased IFN-gamma secretion by CD4+ T-cells ($p<0.05$).
Similar to GF, exposure of IEC to 2'FL alone or 2'FL/GF combined with CpG polarize the immune response towards a Th1 and regulatory type. IEC-derived galectins might be involved in the immunomodulatory effects. moDC exposed to 2'FL and CpG-conditioned IEC instructed Th1 differentiation, suggesting that 2'FL can shape the adaptive immune response by affecting IEC function.

P.C5.04.02

Allergen exposure promotes eosinophil development in murine bone marrow via IL-33/ILC2/IL-5 axis

***E. Winberg**, K. Johansson, C. Malmhäll, J. Weidner, M. Rådinger;*
Institute of Medicine, Gothenburg, Sweden.

Introduction: Type 2 innate lymphoid cells (ILC2s) have been implicated in several inflammatory diseases including asthma. Eosinophilia is a hallmark of allergic airway inflammation where interleukin (IL)-5 is crucial for eosinophil development. The alarmin cytokine IL-33 activates ILC2s to produce IL-5. We investigated the role of IL-33/ILC2/IL-5 axis for eosinophil development in murine bone marrow in IL-33-induced inflammation and allergen-induced airway inflammation.
Methods: Wild type (WT) mice, Rag1 knockout (KO) mice, which lack T and B cells, and lymphocyte deficient Rag2Il2rg KO mice received intranasal doses of recombinant IL-33. Additionally, WT mice received intranasal doses of house dust mite to develop allergic airway inflammation. Flow cytometry was applied for quantification of bone marrow eosinophils and ILC2s. Further, expression of the IL-33 receptor (ST2) was evaluated on bone marrow ILC2s and IL-5+ILC2s were quantified.
Results: Rag1 KO mice demonstrated increased eosinophil numbers and bone marrow ILC2s produced IL-5 *in vitro* similar to WT mice in response to IL-33 challenge. Lymphocyte deficient mice, which lack ILC2s were unable to develop eosinophilic inflammation. Increased levels of ST2 on bone marrow ILC2s were observed for allergen exposed WT mice and correlated with elevated levels of eosinophil progenitors.
Conclusion: Allergen-induced eosinophilic inflammation involves IL-33-responsive bone marrow ILC2s that may support eosinophil development at an early stage. Rag1 KO mice develop bone marrow eosinophilia which demonstrates that adaptive immunity is dispensable for IL-33-driven eosinophil production. Eosinophilia is absent in Rag2Il2rg KO mice which might suggest that IL-5-producing ILC2s are needed for eosinophil induction and development.

P.C5.04.04

Antibody PIPEline by PIPEcloning: Efficient production of Bet-v-1-specific antibodies of different classes sharing the same variable region

***V. K. Köhler**[1,2], J. F. Singer[1,2], K. M. Ilieva[3,4], C. L. Pranger[1,2], S. N. Karagiannis[3,4], E. Jensen-Jarolim[1,2];*
[1]Interuniversity Messerli Institute/Institute of Pathophysiology and Allergy Research, Vienna, Austria, [2]Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria, [3]St. John's Institute of Dermatology, School of Basic & Medical Biosciences, King's College London & NIHR Biomedical Research Centre at Guy's and St. Thomas' Hospital and King's College London, London, United Kingdom, [4]Breast Cancer Now Research Unit, School of Cancer & Pharmaceutical Sciences, King's College London, London, United Kingdom.

The creation of tailor-made antibodies is interesting both in the clinics and in research. PIPE (Polymerase Incomplete Primer Extension) cloning is a cutting-edge method that allows fast assembly of antibody constructs. It furthermore enables the creation of antibodies of several classes sharing the same binding region (Ilieva et al., 2017). We here present PIPE-cloned IgE, IgG_1 and IgG_4 targeting the major birch pollen (Betula verrucosa) allergen Bet v 1.
PIPE cloning was used to create vectors of several antibody classes (IgE, IgG_1, IgG_4) sharing the same variable region against Bet v 1 (Levin et al., 2014). Plasmids were expressed in Expi293F cells and purified with affinity chromatography. Concentration was measured via BCA protein assay and purity and correct assembly was confirmed by SDS PAGE; the specificity of all classes of recombinant antibodies against Bet v 1 was tested in a dot blot, of IgE additionally in an ISAC112 microarray. Affinity chromatography resulted in specific isolation of correctly assembled antibodies, as was confirmed via SDS PAGE. Overall yields were in the range of several hundred micrograms (IgE) to milligrams (IgG_1, IgG_4). All of the produced antibodies specifically bound to Bet v 1 in a dot blot, IgE recognised Bet v 1 in an ISAC112 allergen microarray with high specificity. PIPE cloning is a time-efficient method to obtain a PIPEline for antibodies targeting Bet v 1 for studying class-specific antibody function in type 1 allergy. The work was supported by the Austrian Science Fund FWF grants MCCA W1248-B30SFB and F4606-B28 to EJJ.

P.C5.04.05

Antibody PIPEline by PIPE cloning: Fast production of human monoclonal IgG1, IgG4 and IgE antibodies specific for beta-lactoglobulin from milk

***C. L. Pranger**[1,2], J. F. Singer[1,2], V. K. Köhler[1,2], K. M. Ilieva[3,4], S. N. Karagiannis[3,4], E. Jensen-Jarolim[1,2];*
[1]The interuniversity Messerli Research Institute, Vienna, Austria, [2]Institute of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria, [3]St. John's Institute of Dermatology, School of Basic & Medical Biosciences, King's College London & NIHR Biomedical Research Centre at Guy's and St. Thomas' Hospital and King's College London, London, United Kingdom, [4]Breast Cancer Now Research Unit, School of Cancer & Pharmaceutical Sciences, King's College London, London, United Kingdom.

Background: Immediate type I allergy mediated by specific IgE to milk affects between 1.8 - 7.5% of children during the first year of life (Luyt et al., 2014). Precise tools are missing to establish cellular and molecular models for investigating the role of IgE and, especially IgG1 and IgG4, in milk allergy. Aims: Polymerase Incomplete Primer Extension (PIPE) cloning is the newest approach for the production of monoclonal antibodies (Ilieva et al., 2017). Here we use this method to generate antibodies of diverse subclasses (IgE, IgG1 and IgG4) against the major milk allergen beta-lactoglobulin, termed Bos d 5. Methods: Vectors of antibodies with the variable region against Bos d 5 (Jylhä et al. 2016) were assembled using the PIPE cloning method and transformed into E. coli. Clones were validated by colony-PCR and afterwards expressed in the Expi293F cells. After affinity chromatography, antibody yields were measured with BCA protein assay and recombinant antibodies were controlled by SDS-PAGE for correct assembly and checked for specificity to BLG in dot blot. Results: The recombinant expression yielded 2.2 mg of IgE, 0.6 mg of IgG1 and 1.9 mg of IgG4 antibodies. Correct assembly of all antibodies and their specific binding to BLG was confirmed. Conclusion: In summary, PIPE-cloning enabled us to produce various human antibodies in a short time frame at substantial yields. With the products we aim to contribute important and innovative tools for mechanistic studies on milk allergy. The work was supported by Austrian Science Fund FWF, grants MCCA W1248-B30 and SFB F4606-B28.

P.C5.04.06

T cell responses to sublingual treatment with recombinant Mal d 1

***C. Kitzmueller**, B. Nagl, T. Kinaciyan, B. Bohle;*
Medical University Vienna, Vienna, Austria.

Background: More than 70% of birch pollen-allergic individuals develop birch pollen-related food allergy (BPRFA), most frequently to apple. We recently conducted a clinical study of sublingual immunotherapy (SLIT) with recombinant birch allergen, (r)Bet v 1, apple allergen, rMal d 1, or placebo for 16 weeks. Interestingly, only the patients receiving rMal d 1 significantly improved BPRFA. In the current study, we analysed changes in the T cell compartment of the rMal d-treated patients over the course of treatment. Methods: We investigated T cell reactivity and the expression levels of the key cytokine IL-4, Il-5, IL-13, IFNγ, IL-10 and TGFβ in response to specific stimulation by thymidine incorporation and RT-qPCR, respectively. Additionally, we analysed changes in the relative numbers of $CD4^+$ memory T cell subsets, namely Th1, Th2, Treg and Tfh, with subset-specific surface markers and flow cytometry. Results: The proliferative response to rMal d 1 was reduced over the course of treatment, however, this was not accompanied by changes in the expression levels of cytokines. Almost all of the $CD4^+$ memory T cell subsets analysed were unchanged with the notable exception of pro-allergic Th2 cells ($CD27^-$, $CRTh2^+$, $CCR4^+$), which were significantly decreased already after 4 weeks of treatment. Conclusion: SLIT with recombinant apple allergen shows promising results, however, despite clinical improvements, only minor changes in the T cell compartment were observed. Supported by: OeNB project 16620, the Austrian Science Fund (projects KLI96 and SFBF4610), Biomay AG, and the Christian Doppler Research Association, Vienna, Austria.

P.C5.04.07

EFFECT OF AIR POLLUTION ON PATIENTS WITH BRONVHIAL ASTHMA IN GEORGIA

***R. Sepiashvili**[1], M. Chikhladze[2], S. Gamkrelidze[2];*
[1]Peopels Friendship University of Russia, Moscow, Russian Federation, [2]National Institute of Allergology,Asthma and Immunology of Georgian Academy of Sciences, Tskhaltubo, Georgia.

The study aimed to establish the correlation between the concentration of phadiatop, total IgE levels in the blood in patients with bronchial asthma and the concentration of specific air pollutants in terms of annual calendar of flowering plants in West Georgia. In the study were involved 45 patients (24 males and 21 females) of different ages with bronchial asthma. The study included: I step - allergodiagnostic using "Immuno- CAP 100",
II step - monitoring of aeropollutants concentration by using aeropolinometer "Burkard Trap".Patients had high titers of total IgE, which amounted to an average of 273 (N 33-90), while the average concentration of phadiatop was 96 (N <70).Patients with bronchial asthma of a specific positivity of specific IgE to the weeds (Wx2) - ambrosia, plantain, clasp/tarragon, atriplex -in 25 (55%) on average; tree dust (Tx9) - alder, lactarius piperatus, nuts, oak, willow - 16 (35%) ; and cereals (Gx1) - festuca pratensis, lolium temulentum, timoti grass, poa - 8 (17%); Mx2 -Penicillium notatum, Cladosporium herbarum, Aspergillus fumigatus, Candida albicans, Alternaria alternate- 11 (24%) was revealed , only in 6 (13%) patients we cannot established the allergy specific IgE. From January to April 2017, there were revealed a high concentration of aeropollutants, by high allergenization and widespread; especially high concentrations were found in alder, birch tree and common hazel, while from aeropollutants of low allergenization poplar, elm, willow and plane tree were distinguished. High degree correlation between the above-mentioned markers proves its clinical importance/value with respect to bronchial asthma.
This publication was prepared with the support of the"RUDN University Program 5-100".

P.C5.04.08

In vivo T regulatory cell regulation during human rhinovirus infection

***K. Jansen**[1], O. F. Wirz[1], G. Tan[1], W. van de Veen[1], D. Mirrer[1], S. L. Johnsthon[2], N. G. Papadopoulos[3], C. A. Akdis[1], K. C. Nadeau[4], M. Akdis[1];*
[1]SIAF, Davos Platz, Switzerland, [2]Imperial College London, London, United Kingdom, [3]University of Athens, Athens, Greece, [4]Sean N. Parker Centre for Allergy & Asthma Research - Stanford University, Stanford, United States.

Rationale: Respiratory infections with human rhinoviruses (HRV) are strongly associated with asthma exacerbations and pose a severe health risk for allergic individuals. How HRV infections and chronic allergic diseases are linked, and which role HRV plays in the breaking of allergen-specific tolerance is unknown. T regulatory cells (Tregs) play an important role in the induction and maintenance of immune tolerance. Therefore, the aim of this study is to investigate the effects of HRV on Tregs during asthma exacerbations.Methods: Healthy and asthmatic individuals were experimentally infected with HRV16 in vivo. Peripheral blood mononuclear cells (PBMCs) were obtained before infection and three days after infection. Tregs were sorted from the PBMCs according to their flow cytometric profile CD4+CD3+CD25+CD49d-CD127- and were analyzed with NGS.Results: We have found that upon viral infection in both asthmatics and healthy individuals an antiviral response is induced in Tregs, including upregulation of MX1, STAT1, IFI44L, IRF7/9, OAS3. In healthy individuals there is an additional upregulation of FOS and JUN, and the suppressor molecules SOCS3, CTLA-4 and ICOS, while these were downregulated in asthmatics. Furthermore, in healthy individuals CCL5 was downregulated, while unchanged in asthmatics.Conclusion: Tregs from healthy and asthmatic individuals both show an anti-viral response after RV infection. However there are also clear differences in response between Tregs from healthy and asthmatic individuals. These differences in response might affect Treg functions, level of inflammation, chronicity and viral clearance. Together this data suggest that Treg functions might be altered or impaired during HRV infections, which may contribute to asthma exacerbations.

P.C5.04.09

Qualitative and quantitative comparison of pollen allergen exposure between horses and humans. A collaborative study with the Austrian Pollen Information Service

***A. D. J. Korath**[1], K. Bastl[2], G. Hofstetter[1], K. Hufnagel[1], M. Kmenta[2], U. E. Berger[2], E. Jensen-Jarolim[1,3];*
[1]1) The Interuniversity Messerli Research Institute, University of Veterinary Medicine Vienna, Medica, Vienna, Austria, [2]2) Medical University of Vienna Department of Oto-Rhino-Laryngology Aerobiology and Pollen Information Research Unit, Vienna, Austria, [3]3) Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria.

Background: In our recent study we revealed that IgE patterns in ISAC allergen microarray (Einhorn et al, Allergy 2018) differed between horses and humans, suggesting different allergen exposure.
Aims: We therefore aimed to collect pollen and plants from horse paddocks and meadows and compare to the pollen counts assessed for humans by the Austrian pollen Information Service.
Methods: Pollen were collected, on paddocks and pastures in four different horse stables, using pollen traps in April and in June 2018. The surrounding vegetation was examined botanically to correlate the pollen with the occurrence of allergenic plants.
Results: The overall pollen count was higher in early summer. In spring mostly pollen from Picea, Quercus and Fagus were found and in summer Poaceae, Urticaceae, Sambucus and Plantago pollen. The same pollen species as relevant for humans occur in the equine environment, however, largely differing in terms of quantitative composition.
Conclusions: This study will provide evidence whether the human APIS (Austrian Pollen Information Service) can be useful for owners of sensitized horses.
This study is supported by Austrian Science Fund FWF grant SFB F4606-B28 to EJJ.

P.C5.04.10

Qualitative multiplex detection of allergen-specific IgG and IgA on a microarray

***G. Feyzkhanova**[1], O. Smoldovskaya[1], S. Voloshin[1], M. Filippova[1], E. Antonova[2], L. Pavlushkina[2], T. Filatova[2], A. Rubina[1];*
[1]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russian Federation, [2]Filatov Moscow City Pediatric Clinic No. 13, Moscow, Russian Federation.

Allergen specific IgE (sIgE) is the main marker of the hypersensitivity type I in atopic individuals. However, in certain cases adverse reactions or their absence cannot be explained only via IgE diagnostics. Detection of allergen-specific immunoglobulins of other classes, such as specific IgG (sIgG) or specific IgA (sIgA), and specification of their role in allergic disorders can give additional information that might improve allergy diagnostics.
For that purpose a microarray for multiplex qualitative determination of sIgG and sIgA to 31 allergens was developed. Microarray includes semispherical gel pads containing allergen extracts or individual proteins belonging to different groups: pollen, indoor or food allergens. 10µl of the blood serum is necessary for the analysis. After the incubation of microarray with diluted blood serum (1:15) microarray is developed with anti-human antibodies conjugated with fluorescent dyes.
sIgG and sIgA profiles were obtained on the microarrays for 30 patients with different allergic diseases, using as developing antibodies anti-IgG-Cy3, anti-IgA-Cy5 or the mix of these antibodies. The signals for individual antibodies and for their mixture agreed within standard deviation of the analysis, which indicates that the developing system was selected properly. Also, the obtained patterns of sIgG and sIgA responses didn't coincide with the pattern of sIgE response defined previously.
The assay procedure developed is simple and may be used for screening both in adults and children to specify the relation between immunoglobulins G and A against allergens of different origin.
This work was supported by the Russian Science Foundation, Grant No. 14-50-00060.

P.C5.04.11

Maternal exposure to antibiotics increases asthma severity in offspring mice

I. Lingel[1], *J. Gray*[2], *H. Deshmukh*[2], *P. Koenig*[1], *I. Lewkowich*[3];
[1]*Institute of Anatomy, Luebeck, Germany,* [2]*Division of Neonatology, Perinatal and Pulmonary Biology, Cincinnati Children´s Medical Center, Cincinnati, United States,* [3]*Division of Immunobiology, Cincinnati Children´s Medical Center, Cincinnati, United States.*

Within the last 40 years, asthma prevalence has tripled. As genetics do not change this rapidly, it is thought that recent changes in life-style in developed nations is increasing the risk of developing allergic asthma in genetic susceptible individuals. Recent epidemiological studies link early life exposure to antibiotics or other microbiota-changing events with an increased risk of the child to develop asthma. In this study, we seek to understand the role of early life microbiota disruption on asthma pathogenesis in mice. Offspring of mothers exposed to antibiotics (ABX) in drinking water (1mg/mL of Ampicillin, Gentamicin and Vancomycin) from embryonic day 15 (E15) to postnatal day 14 (P14) displayed a markedly exacerbated allergen-induced asthmatic phenotype with high airway hyperresponsiveness (AHR), increased numbers of IL-17A^{+} innate lymphoid cells (ILCs), neutrophilia, and high levels of IL-22 in the bronchial lavage. A more limited ABX exposure regimen (day E15 to birth) was associated with less severe exacerbation of AHR, recruitment of ILC3s, but had limited impact on airway neutrophilia and BAL cytokine levels. Interestingly, the limited ABX exposure regimen was also associated with alveolar enlargement, and increased airway permeability. These observations suggest that early microbial exposures in critical windows after birth limit pathogenic immune responses in a sequential manner, and suggests that proper microbial colonization might have a previously unrecognized role in promoting normal lung maturation. Disrupting such colonizations may cause inadequate immune responses later in life.

P.C5.04.12

Immunoglobulin heavy-chain repertoire profiling of memory B cell, plasmablasts and plasma cells from peripheral blood of individuals with birch pollen allergy

A. I. Mikelov[1], *M. A. Turchaninova*[2], *E. A. Komech*[2], *E. S. Egorov*[2], *D. B. Staroverov*[2], *Y. B. Lebedev*[2], *D. M. Chudakov*[2,1], ***I. V. Zvyagin***[2,3];
[1]*Skolkovo Institute of Science and Technology, Moscow, Russian Federation,* [2]*Shemiakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russia, Moscow, Russian Federation,* [3]*Dmitry Rogachev National Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation.*

Mechanisms underlying allergy development and maintenance still remain obscure. IgE produced by B-cell lineage cells is the known causative agent triggering clinical manifestations. The cell subsets, responsible for production of IgE and its persistence in human body are not well characterised. Little is known about structure and seasonal dynamics of B-cell repertoire of allergic individuals. In this study we aimed to characterize IGH repertoires of key cell subsets of B-cell lineage from the peripheral blood of donors, susceptible to birch pollen allergy.
Using state-of-the-art technique for IGH library preparation which allows molecular-barcode based error correction and data normalisation, we studied the full-length IGH repertoires in several time points during 1 year, including of-season and peak of birch pollen periods. Clonal groups with IgE clonal sequence were detected in 3 of 4 allergic and 1 of 2 healthy donors. In contrast to IGH repertoires of healthy donors, we found IgE-containing clonal groups in repertoires of multiple cell subsets of allergic individuals. Such clonal groups also contained IgG, IgM and IgA members, and persisted in several time points. In memory B cells of 4 out of 6 donors we found clonal sequences of IgE isotype. These IGH clonotypes were hypermutated at the rates, similar to those of IgG and IgA clonotypes from IgE-containing clonal groups.
The study was supported by Council on Grants of Russian Federation President (grant #MK6000.2018.4 to ZIV) and Foundation for Assistance to Small Innovative Enterprises in Science and Technology (UMNIK grant #10861GU2016(0024432) to MAI).

P.C6.01 Innate control of inflammation and tissue repair - Part 1

P.C6.01.01

Immunomodulation effect of biosynthetic dressing loaded with silver nanoparticles and TNF inhibitor in a non-traumatic wounds experimental model

A. Aguilar-Hernandez[1], *N. Garcia-Becerra*[1], *L. Fernandez-Avila*[2], *A. Zamudio-Ojeda*[2], *D. A. Lopez-de la Mora*[3];
[1]*Universidad de Guadalajara- UAG, Guadalajara, Mexico,* [2]*Universidad de Guadalajara, Guadalajara, Mexico,* [3]*Universidad de Guadalajara- LAMAR, Guadalajara, Mexico.*

The chronic wounds are stopped in the inflammatory phase, thus preventing the regeneration of the same. Silver-like nanoparticles have antibacterial properties, these have been used in a wide range of biomedical applications due to their selective toxicity in microorganisms and low immunogenicity. In addition, the use of immunomodulators contributes to inflammatory regulation to promote healing and regeneration.
Aim. Formulate a biosynthetic dressing loaded with silver nanoparticles and a TNFa inhibitor that promotes tissue regeneration and bacterial inhibition in an experimental model of chronic wounds.
Methodology. Chitosan gel was formulated. Silver nanoparticles were synthesized and their size was corroborated by SEM, TEM and IR. Dissolution time tests of the pharmaceutical form were carried out according to the Mexican pharmacopoeia. A bacterial inhibition test was performed in solid culture media type antibiogram. An experimental model of chronic wounds was established. Tissue histologies were performed. The genes of TNFa, TGFb1, MMP8, IL-1 and IL-17 were measured.
Results. Chitosan with 90% purity was obtained. Subsequently, the chitosan was solubilized until it became semi-solid. Silver nanoparticles of 30 nm were synthesized by chemical synthesis. A formula was standardized with previous compounds and a TNFa inhibitor. In the solubility tests, formula was kept releasing drug for 5 days at different pH. Bacterial inhibition was achieved by visualizing agar bacteria inhibition. The inflammation was decreased histologically. As well as a decrease in the expression of proinflammatory cytokines was observed in the treated group. Healing of the wound was achieved before 15 days compared to the negative control.

P.C6.01.02

HIV-induces IL-18 from intestinal epithelial cells that causes cell death, increased intestinal permeability & microbial translocation

A. Ahmad*, O. Allam, S. Samarani;*
CHU Ste-Justine/University of Montreal, Montreal, Canada.

IL-18 is a pro-inflammatory cytokine belonging to the IL-1 family. It has been shown that HIV infection is accompanied by an imbalance in the production of IL-18 and of its natural antagonist, the IL-18 Binding Protein (IL-18BP). The infection is also accompanied by intestinal inflammation, increased intestinal permeability and microbial translocation. However, little is known concerning the potential role of the cytokine in the causation of the intestinal pathology. Here we show that incubation of HIV with human intestinal epithelial cells (IEC) increases production of IL-18 and decreases that of IL-18BP from the IEC. The cytokine induces apoptosis in IEC in a time and dose dependent manner via activating caspase-1 and -3. It modulates the expression of several Tight and Adherens Junction proteins e.g., occludin, claudin 2 and β-catenin. It also disorganizes F-actin expression in the IEC. The cytokine decreases transepithelial electrical resistance (TEER) and increases permeability in the IEC monolayers. It also increases the expression of phosphorylated myosin II regulatory light-chain (p-MLC) and myosin light-chain kinase (MLCK), and decreases activation of STAT-5 in the IEC. A Rho-kinase (ROCK)-specific inhibitor suppresses the cytokine-induced increase in p-MLC. Interestingly, the levels of the cytokine correlate with those of LPS in the circulation in HAART-naïve and HAART-treated HIV-infected individuals, Elite controls as well as in healthy controls. Taken together, our results suggest that the increased concentrations of IL-18 play a role in increased intestinal permeability and microbial translocation observed in HIV-infected individuals. Thus targeting the cytokine may ameliorate intestinal pathology in HIV-infected individuals.

P.C6.01.03

Higher frequencies of lymphocytes expressing the natural killer group 2D receptor and cytotoxic potential of NK cells in patients with Behcet disease

M. Bonacini*, A. Soriano, Z. Alessandro, E. Calò, L. Cimino, F. Muratore, L. Fontana, M. Parmeggiani, C. Salvarani, S. Croci;*
Azienda Unità Sanitaria Locale-IRCCS di Reggio Emilia, Reggio Emilia, Italy.

Aim: This study aimed to identify a specific profile of circulating Natural Killer (NK), NKT and T cells able to discriminate patients with Behçet disease (BD) and Healthy controls (HC). **Methods:** Peripheral blood mononuclear cells (PBMCs) were collected from 38 BD patients and 15 HC. The frequencies of NK, NKT and T cells expressing CD16, CD69, NKG2D, Nkp30, Nkp46 and NKG2A were assessed by flow cytometry. Cytotoxic potential of NK cells was evaluated by flow cytometry as the percentage of cells expressing the degranulation marker CD107a after incubation with K562 cells. The levels of 27 cytokines were determined in plasma with a multiplex bead-based assay. **Results:** Higher percentages of NK, NKT and T cells expressing NKG2D were detected in PBMCs of BD patients than HC. ROC curve analysis showed that the evaluation of NKG2D^{pos} NK, NKT and T cell percentages discriminated between BD patients and HC. Moreover, there was a positive correlation between the BD Current Activity Form (BDCAF) scores and the frequencies of NKG2D^{pos} NK and NKT cells. A higher frequency of NK cells expressing CD107a was induced in PBMC from BD patients than HC after incubation with K562 cells. Concentrations of IL-5, IL-10, IL-12, IL-13, IP-10 and MIP-1β were higher in plasma of BD patients than HC. **Conclusion:** monitoring the frequencies of NKG2D^{pos} lymphocytes, and CD107a^{pos} NK cells after the degranulation assay could help the clinicians in BD patients management. The increased expression of NKG2D in BD patients is likely involved in disease pathogenesis.

P.C6.01.04

Evolutionary conserved cell- and immunobiological processes during tissue regeneration: comparative studies in an invertebrate model

*K. Bodó[1], É. Rumpler[2], B. Kokhanyuk[1], P. Németh[1], **P. Engelmann**[1];*
[1]Department of Immunology and Biotechnology, Clinical Center, Medical School, University of Pécs, Pécs, Hungary, [2]Department of Comparative Anatomy and Developmental Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary.

It is well known that annelid (segmented) worms possess strong regeneration capacity. However limited information is available about the interactions of regeneration and immune-related mechanisms in earthworms. Our aim was to compare cell proliferation vs. cell death and the expression of certain immune-related genes in the course of anterior/posterior regeneration of the earthworm *Eisenia andrei.* To enumerate cell proliferation Click-iT EdU assay was applied following 2 and 4 weeks of regeneration. It was combined with FITC-coupled anti-EFCC mAbs to assess the involvement of different immunocytes (e.g. coelomocyte) subsets in the restoration process. Apoptotic activity was detected by TUNEL assay. Q-PCR analysis was executed to evaluate the expression of mRNA targets in the regenerating segments of *E. andrei*. A high number of proliferating cells were detected after 2 weeks that is decreased by the 4 weeks only in the posterior blastema. In contrast, the apoptotic activity was observed throughout the restoration. Immunostaining revealed that coelomocyte subsets were accumulated in the blastema mainly during the posterior regeneration. Pattern recognition receptor genes evidenced a decreased pattern (except scavenger receptor) compared to intact animals. Several genes have similar expression pattern (TLR, LBP) during anterior/posterior regeneration, except the antimicrobial molecules (lysozyme). Evaluation of cell biological events and immune-related gene expression revealed characteristic differences during anterior/posterior regeneration that is a novel observation in the field of invertebrate (earthworm) immunity. This work was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and Medical School Research Foundation, University of Pécs.

P.C6.01.05

Soluble triggering receptor expressed on myelocytes -1 is strongly correlated with disease activity in systemic lupus erythematous

***I. Gkougkourelas**, A. Gkantaras, A. Georgiadou, P. Boura;*
Clinical Immunology Unit, 2nd Internal Medicine Department, Hippokration General Hospital, Aristotle University of Thessaloniki, Thessaloniki, Greece.

Background: Soluble Triggering Receptor Expressed on Myelocytes -1 (sTREM-1) is an innate immunity receptor, which participates in inflammatory reactions. Its serum levels reflect the magnitude of systemic inflammatory response and can discriminate between infectious and non-infectious causes. Its role in systemic lupus erythematosus (SLE) is unknown. In this study we examined sTREM-1 in SLE patients with regard to disease activity. **Patients and Methods:** Sixteen patients with SLE were enrolled. Diagnosis was based on the revised 1997 American College of Rheumatology criteria. Disease activity was measured with the SLE Disease Activity Index-2000 (SLEDAI-2K). sTREM-1 levels were determined by Enzyme Linked Immunosorbent Assay (ELISA) in serum samples. Seventeen age- and sex-matched healthy individuals comprised the control group. Statistical analysis was performed with the SPSS package; $p<0.05$ was considered significant. **Results:** Serum sTREM-1 was significantly higher in SLE patients than in normal controls. Its levels were strongly correlated to SLEDAI-2K. **Conclusions:** Serum sTREM-1 is strongly correlated to disease activity in SLE, probably reflecting the generalized activation of the innate immunity response in this disease and may be an additional biomarker in SLE. **Conflict of interest** None **Acknowledgements** To IKY fellowships of excellence in postgraduate studies in Greece -Siemens program

P.C6.01.06

Investigating the innate training potential of the vaccine adjuvant alum

***A. L. Gorman**, E. C. Lavelle;*
Trinity Biomedical Sciences Institute, Dublin, Ireland.

Vertebrate immunity is classically divided into innate and adaptive immune responses. Recent findings have challenged the classical view of innate versus adaptive immunity, suggesting that innate cells can retain some "memory" of past immunological insults. This "trained immunity" which allows for primed cellular responses to secondary infections is independent of T and B cells and is mediated by innate cells such as monocytes/macrophages and NK cells. While it has been shown that certain live vaccines such as BCG can induce trained immunity, the effects of particulates such as the widely used vaccine adjuvant alum have not been addressed. Alum has been predominantly used in many vaccines due to its humoral immunopotentiation and safety profile.
However, how this widely used adjuvant elicits its effects remains elusive. We have shown that alum-trained macrophages adopt a distinct morphology in addition to an altered capacity for cytokine secretion upon re-stimulation with lipopolysaccharide *in vitro*. Using a NanoString inflammation panel to characterize the transcriptional profile of alum-trained cells we have found that training by this adjuvant globally downregulates proinflammatory genes. Further investigation into the *in vitro* effects of alum-trained macrophages will help shed light on the mechanism by which this particulate exerts its innate training effects in macrophages.

P.C6.01.07

Complement C3 and C4 are useful markers for predicting Bipolar disease severity and monitoring patients under psychotropic treatment

***H. Hachicha**[1], N. Halouani[2], R. Feki[2], S. Feki[1], F. Ayadi[1], A. Maatoug[1], A. Ayadi[1], S. B.Hamadou[1], j. Aloulou[2], H. Masmoudi[1];*
[1]Immunology Department, CHU Habib Bourguiba, Sfax, Tunisia, [2]Psychiatric department, CHU hedi Chaker, Sfax, Tunisia.

Abnormalities of the immune system have recently been shown to be implicated in bipolar disease (BD). BD patients have been also reported to exhibit increased proinflammatory cytokine levels indicating the role of inflammation in this disease. In our study we aimed to find out whether complement system (C3 and C4 fractions) and immunoglobulins (Ig) are abnormal in BD and to explore the effect of psychotropic treatment on these variables This study was conducted during 36 months on 90 subjects (45 patients with manic relapse in type I BD (DSM 5 criteria) having no history of autoimmune disease and 45 age and sex matched controls. Mean plasma IgG and complement C3 levels were significantly higher in bipolar patients with manic relapse (p = 0.034, p = 0.040). The plasma level of the C3 and C4 fraction was correlated with EGF score (r = 0.375, p = 0.016, r = 0.340, p = 0.016). After treatment, there was a statistically significant increase in mean plasma IgG and IgA levels (p = 0.006, p = 0.009) and a decrease in the mean plasma C4 complement level (p = 0.004). Mean plasma IgM levels were significantly lower on sodium valproate treated patients (p = 0.004). Under atypical antipsychotics, the mean plasma level of the C3 fraction was statistically lower (p = 0.028) whereas under conventional antipsychotic it was statistically higher (p = 0.048). Among the variables studied, the complement system seems to be closely related to manic relapse, its severity, and psychotropic treatment.

P.C6.01.09

Assessment of autophage function in systemic lupus erythematosus in respect of hyperlipidemia and immunosuppressive drugs

***A. S. Hamada**[1], m. i. aref[1], a. m. rabee[2];*
[1]Al Azhar university hospital, cairo, Egypt, [2]aswan university hospital, aswan, Egypt.

Background: Autophagy is an orchestrated homeostatic process to eliminate unwanted proteins and damaged organelles in addition to regulation of lipids.
Objective: Assessment of autophagy focusing on lipids regulation in patients with SLE .
Patients and Methods: Subjects were divided into three groups. Group 1 included 60 newly diagnosed SLE patients before receiving any treatment, group2 included the same subjects of group 1 after three months of treatment with immunosuppressive drugs and group 3 included 30 matched healthy donors as a control group. Disease activity was assessed by (SLEDAI) score, lipid profile was measured in addition to evaluation of lipids uptake, enhanced phagocytosis and intracellular killing ability of monocytes and neutrophils using Sudan Black B & Nitroblue tetrazolium stains.
Results:There was a positive correlation between total cholesterol, LDL and triglycerides and disease activity(SLEDAI score) (r= 0.677, r= 0.603 and r= 0.718; respectively).On the contrary, There was a negative correlation between HDL and disease activity(r= -0.396). Furthermore, there was a negative correlation between lipid content of cells and intracellular killing and disease activity(r= -0.258 and r= -0.324; respectively) . After 3 months, comparing group 2 to group 1, there was significant increase in cholesterol, LDL and trigycerides (P=0.027, P=0.021 and P=0.017; respectively) while HDL showed insignificant difference(P=0.0740). Lipid content in cells and intracellular killing significantly decreased(P=0.0322 and P=0.0271; respectively).
Conclusion: Autophagy is deficient in patients with SLE so they are more susceptible to infections and dyslipidemia, aggravated by immunosuppressive drugs. Consequently, lipid lowering drugs are definitely required to decrease comorbidity .

P.C6.01.10

The "Who is Who" of dermal infiltration in fibrosis developing *fos- related antigen- 2* mice

***J. Haub**[1,2], V. K. Raker[1,2], N. Lorenz[1,2], F. Steinbach[3,2], E. Wagner[4], D. Schuppan[3,2], K. Steinbrink[1,2];*
[1]Department of Dermatology, Mainz, Germany, [2]Research Center for Immunotherapy, University Medical Center Mainz, Germany, [3]Institute of Translational Immunology, Mainz, Germany, [4]National Cancer Research Center, Madrid, Spain.

Systemic sclerosis is a complex and incompletely understood disease, resulting in skin and organ fibrosis. Cutaneous fibrosis is characterized by disproportionate accumulation of collagens and other extracellular matrix substances. However, involvement of innate immune cells and the sequence of inflammatory events in the early inflammatory phase of the disease have not been addressed so far.

Therefore we studied the murine model of systemic sclerosis: The *fos-related antigen-2* mouse (fra-2). Fra-2 mice overexpressing fra-2 under MHCI promotor control and spontaneously developing vasculopathy and fibrosis in the skin and internal organs. In Fra-2 mice the inflammatory response peaks at week 7-9 of life. By week 12 mice develop massive dermal and pulmonary fibrosis leading to dyspnea by week 17.
At week 12 histological analysis of Fra-2 mice showed a significant enhancement of dermal thickness and a pronounced cutaneous collagen accumulation (H&E, Goldner´s trichrome, hydroxyproline assay).
Flow cytometric analysis of the blood and skin demonstrated an early cellular infiltrate consisting of activated myeloid cells (Ly6C$^+$MHCII$^+$). This myeloid infiltration peaked at week 9 and comprised CD11b$^+$CD301b$^+$ as well as CD11b$^+$CD206$^+$ cells, indicating an infiltration of terminally differentiated macrophages in Scl-prone skin.
Along with myeloid cells Fra-2 mice exhibit pronounced levels of activated T-cells (CD4$^+$CD25$^+$), but a reduced proportion of CD4$^+$Foxp3$^+$ regulatory T-cells in the skin, lymph nodes, lung.
Our findings suggest that reduced Treg frequencies accompanied by a massive myeloid and lymphoid infiltration contribute to fibrosis development in Fra-2 mice.
This study is financially supported by the German Research Foundation (Collaborative Research Center (CRC/TR156).

P.C6.01.11
2B4 RECEPTOR REGULATE COLITIS DEVELOPMENT VIA TISSUE REPAIR MECHANISM.

***T. Kim**, J. Kim, B. Park, G. Park, S. Lim, K. Lee;*
Korea University College of Medicine, Seoul, Korea, Republic of.

Ulcerative colitis is a chronic relapsing form of inflammatory bowel disease (IBD) that causes inflammation and ulcers in the colon. Innate immune responses are important in the initiation and progression of inflammatory bowel disease (IBD). 2B4 (CD244), a member of SLAM family play a crucial role in inflammatory disease. 2B4 is expressed by a large number of innate immune cells in intestinal lamina propria (LP). However, the role of 2B4 receptor in intestinal inflammation is still unknown.
Since 2B4 is highly expressed on some of lamina propria lymphocyte (LPL) in intestine, we hypothesized that 2B4 might play and crucial role for intestinal inflammation. Indeed, we found that 2B4 is expressed dendritic cells, macrophage, ILCs in intestinal lamina propria, and upregulated expression level after DSS administration. The progression and disease score of intestinal inflammation induced by DSS in 2B4$^{-/-}$ mice was much faster and severe than that observed in wild-type mice. At the cellular and tissue level, we found increased colonic inflammation and reduced intestinal epithelial cell proliferation, along with severely diminished number of dendritic cells in colon. Tissue repair related cytokines (IL-22, IL-18) are significantly reduced in 2B4 deficient mice, and replenishing these cytokine could recover colonic inflammation in 2B4 deficient mice group. Taken together, these data identify a novel function of CD244 as a gate guard in intestinal inflammation in IBD
This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (Grant#; NRF-2016R1A6A3A04009698 and NRF-2017R1A2B3004828)

P.C6.01.12
ADSCs treatment hinders the advancement of fibrosis in liver in the early stage of NASH by reducing the Il17a-mediated inflammation

***A. Nasti**[1], Y. Sakai[2], M. Yamato[1,2], K. Kawaguchi[2], T. Ho[2], K. Yoshida[1], T. Komura[1], M. Takamura[1,3], T. Wada[1,4], M. Honda[2], S. Kaneko[1,2];*
[1]System Biology, Kanazawa University, Kanazawa, Japan, [2]Department of Gastroenterology, Kanazawa University Hospital, Kanazawa, Japan, [3]Department of Cardiology, Kanazawa University Hospital, Kanazawa, Japan, [4]Department of Laboratory Medicine, Kanazawa University, Kanazawa, Japan.

Non-alcoholic steatohepatitis (NASH) liver is defined by a steatotic/inflamed condition with advancing fibrosis, eventually becoming cirrhotic. There is no established therapy and pathogenesis is not completely uncovered. We demonstrated previously that adipose tissue derived stromal/stem cells (ADSCs) decreased fibrosis in NASH mice, now we investigated the mechanism between NASH and simple steatosis and, the effect of ADSCs treatment on early stage of NASH. C57Bl/6J mice were used for ADSCs isolation; simple steatosis or NASH was established in mice and hepatic inflammatory cells (HIC) isolated for DNA microarray analysis and cytokine secretion assay. Anti-Il17a or anti-CD4 antibody was administered weekly for 8 weeks, liver tissues isolated on week 9. Separately, ADSCs were administered on weeks 4 and 8, then liver collected on week 12. Fibrosis was assessed by AZAN staining, FACS and qRT-PCR. DNA microarray of HIC highlighted 868 up-regulated genes in NASH compared to simple steatosis, biological processes were associated to CD4$^+$ T-cell immune response. Frequency of Il17-secreting cells in NASH-HIC was higher (37%) than control (15%). Anti-Il17a antibody decreased fibrosis ($p<0.01$), and down-regulated *Col4a1* in NASH livers ($p<0.05$), anti-CD4 antibody was not effective. Administered ADSCs decreased *Il17a* expression in NASH-HIC, reduced the Il17a-secreting cells population in HIC (25%) compared to control (37%), decreased fibrosis ($p<0.01$) and expressions of *Col4a1* and *Col1a1* in liver tissue ($p<0.05$). In conclusion, Il17a is involved in fibrosis development in NASH mice, and ADSCs administration reduced fibrosis by suppressing the Il17a-mediated inflammation in early stage of NASH.

P.C6.01.13
Differential innate immunity signature associated with disease activity in autoimmune diseases: systemic lupus erythematosus, rheumatoid arthritis, and systemic sclerosis

***A. Petrackova**[1], A. Smrzova[1], M. Schubertova[1], M. Skacelova[1], R. Fillerova[1], V. Smotkova Kraiczova[1], M. Radvansky[2], M. Kudelka[2], F. Mrazek[1], P. Horak[1], E. Kriegova[1];*
[1]Faculty of Medicine and Dentistry, Palacky University and Hospital Olomouc, Olomouc, Czech Republic, [2]Technical University of Ostrava, Ostrava, Czech Republic.

Introduction: Mounting evidence indicates that innate immunity, especially Toll-like-receptors (TLR) and interleukin (IL)-1/IL-1R families, play essential roles in the pathogenesis of autoimmune diseases. The differential innate expression pattern associated with disease activity in systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and systemic sclerosis (SSc) has not been established. Objective of the study was to elucidate the underlying differences in innate immunity signatures associated with disease activity in major autoimmune diseases.
Methods: We investigated gene expression of *TLR1-10*, 7 members of *IL-1/IL-1R* family, and interleukin 8 (*IL-8/CXCL8*) in peripheral blood mononuclear cells from patients with autoimmune disorders taken at time of active disease: SLE (n=28, SLEDAI>6), RA (n=36, DAS28≥3.2), and SSc (n=22, revised EUSTAR index>2.25) using high-throughput SmartChip Real-Time-qPCR system (WaferGen). Statistics were performed by R statistical software, *P*-value<0.05 was considered as significant.
Results: RA differed from SLE and SSc by the upregulated expression of six genes (*TLR2*, *TLR3*, *TLR5*, *SIGIRR*, *IL-1RAP*, and *IL-18R1*; *P*<0.05). Active SLE and SSc showed high similarity in term of immunity gene expression signatures. In SSc, downregulated expression of *IL-18R1* (*P*<0.05) was observed when compared to SLE and RA. In SLE, downregulated expression of *IL-1R1* (*P*<0.05) was detected when compared to RA and upregulation of *IL-18R1* (*P*<0.05) when compared to SSc.
Conclusions: Innate immune gene expression signature in patients with autoimmune diseases in active disease stage was identified, showing high similarity between SLE and SSc.
Grant support: MZ CR VES15-28659A, IGA UP_2018_016, MH CZ - DRO (FNOL, 00098892)

P.C6.01.14
Systemic inflammation induced by administration of TLR-7/8 agonist Resiquimod causes severe thrombocytopenia, myocardial erythrocyte accumulation and iron deposition in CFN mice

*N. Baxan[1], A. Papanikolaou[2], I. Salles-Crawley[3], R. Chowdhury[2], O. Dubois[1], N. Rosenthal[2], L. Zhao[1], S. E. Harding[2], **S. Sattler**[2];*
[1]Imperial College London, Biological Imaging Centre, London, United Kingdom, [2]Imperial College London, NHLI, London, United Kingdom, [3]Imperial College London, Centre for Haematology, London, United Kingdom.

Introduction: Topical application of the TLR-7/8 agonist Resiquimod has been suggested as inducible model of systemic lupus erythematosus. In response to Resiquimod administration, CFN (C57Bl/6xFVBxNOD) mice develop acute myocarditis accompanied by myocardial haemorrhaging, followed by fibrosis and development towards inflammatory dilated cardiomyopathy. Importantly, we have shown previously that CFN mice show a degree of Resiquimod-induced cardiac damage which is significantly more severe than the levels observed in all three parental strains. **Methods:** CFN mice were treated with topical application of Resiquimod to the ear three times a week for two weeks. Magnetic resonance imaging was performed *in vivo*, blood collected for analysis of platelet count and hearts collected for histological analysis of inflammatory damage and fibrosis. **Results and conclusion:** Magnetic resonance imaging shows a significant drop in T_2^* relaxation times (3.7+/-0.17ms and 2.75+/-0.29ms; p-value 0.014, n=3-5), localized primarily in the subepicardial regions of the intraventricular septum of Resiquimod-treated mice, suggestive of the presence of paramagnetic iron. This observation was confirmed by staining for iron in corresponding histology sections, which showed a significant iron accumulation (score: 0.096+/-0.07 and 1.36+/-0.22; p-value 0.005, n=3-10) in areas of low T2* values. We also detect severe thrombocytopenia in Resiquimod-treated CFN mice (platelet count/ul 700+/-132x103 and 17+/-16x103; p-value 0.033, n=3), suggesting that cardiac erythrocyte accumulation may be caused by acute bleeding problems and that haemoglobin is the source of interstitial iron. **Acknowledgements:** This work was supported by the British Heart Foundation RM/13/1/30157 to SEH and PG/16/93/32345 to SS.

P.C6.01.15

Investigating neutrophil impairment in patients with Neuromyelitis Optica

M. Schroeder Castagno[1], *S. Romero Suarez*[1], *N. Borisow*[2], *F. Paul*[3], *C. Infante Duarte*[1];
[1]*Institute for Medical Immunology, Experimental Neuroimmunology, Charité - Universitätsmedizin Berlin, Berlin, Germany,* [2]*NeuroCure Clinical Research Center NCRC, Clinical Neuroimmunology, Charité - Universitätsmedizin Berlin, Germany, Berlin, Germany,* [3]*NeuroCure Clinical Research Center NCRC, Clinical Neuroimmunology, Charité - Universitätsmedizin Berlin, Department of Neurology, Charité - Universitätsmedizin Berlin, Germany, Berlin, Germany.*

Introduction: Our group has recently shown that peripheral blood neutrophils of patients with neuromyelitis optica (NMO) show a deficient respond to migratory and oxidative burst stimuli when compared compared to Multiple Sclerosis (MS). It is well established that in contrast to the observations in MS, neutrophils accumulate in NMO lesions and contribute to the damage cascade inside the CNS. However, it remains unclear, which factors lead to this accumulation. Defective neutrophil apoptosis accompanied by neutrophil accumulation promoting a pro-inflammatory environment has been already shown for other chronic inflammatory diseases. Thus, we here hypothesize that a generalized impaired cell death of NMO neutrophils might support their accumulation in inflammatory lesions.
Methods: We performed an assay to monitor death susceptibility of peripheral blood neutrophils from 20 NMO patients as well as 20 sex and gender matched healthy controls. Neutrophilic death was induced by incubating purified granulocytes with 25 nM PMA for 30 min at 37 °C. Cell death was analyzed by flow cytometry in CD16b$^+$ neutrophils using 7- AAD and Annexin V staining.
Results: Neutrophils from NMO patients show reduced cell death susceptibility compared to HC in our in vitro set-up.
Conclusion: Impaired cell death of neutrophil from NMO patients may contribute to neutrophil pathological accumulation in lesions. However, since the majority of the NMO patients are under immunomodulatory treatments, further evidences from untreated patients are needed to support this conclusion.

P.C6.01.16

Development and characterisation of a 4-dimensional *in vitro* model of ANCA-associated vasculitis

C. A. Walls[1], *N. Basu*[2], *L. P. Erwig*[1], *D. Kidder*[3];
[1]*Institute of Medical Sciences, Aberdeen, United Kingdom,* [2]*Institute of Applied Health Sciences, Aberdeen, United Kingdom,* [3]*NHS Grampian, Aberdeen, United Kingdom.*

Introduction: ANCA-associated vasculitis (AAV) is a group of devastating autoimmune diseases affecting small/medium sized blood vessels. The interaction of neutrophils and monocytes with the endothelial lining of blood vessels is key to understanding disease pathophysiology. There is limited knowledge about the temporal dynamics of these interactions and how they alter throughout the course of disease. We therefore aimed to develop a 4-dimensional *in vitro* model of AAV which would allow investigation of these crucial leukocyte-endothelial interactions.
Materials and Methods: Neutrophils and monocytes were isolated from peripheral venous blood collected from patients with AAV (Granulomatosis with Polyangiitis, Microscopic Polyangiitis and Eosinophillic Granulomatosis with Polyangiitis) and healthy donors. Using live cell spinning disc confocal microscopy, neutrophil/monocyte-human umbilical vein endothelial cell interactions were imaged in three dimensions for 3 hours. Video analysis software (Volocity 6.3) enabled quantification of leukocyte migration, adhesion, transmigration and degranulation.
Results: Degranulation and transmigration was significantly higher in neutrophils isolated from patients with active disease, compared to those in remission and healthy donors. Transcellular, rather than paracellular, transmigration of monocytes was significantly increased in the ANCA-positive patient cohort, compared to those who were ANCA-negative and healthy donors.
Conclusion: We have developed a novel 4-dimensional *in vitro* model of AAV encompassing several crucial leukocyte-endothelial functions at the forefront of disease pathophysiology. These preliminary experiments have highlighted key areas which are likely to be crucial in the development of the disease. Future work will include collecting longitudinal patient samples which will further verify the findings of this model.

P.C6.01.17

Epithelial IL-33 is regulated by the lymphoid stress-surveillance (LSS) response and confers protection against skin carcinogenesis

S. Ward, *R. Castro Seone, G. Crawford, M. Hayes, J. Strid;*
Imperial College London, London, United Kingdom.

Lymphoid stress-surveillance (LSS) refers to the capacity of tissue resident intraepithelial lymphocytes (IEL) to directly sense epithelial cell (EC) dysregulation and initiate a restorative response. γδTCR$^+$ IEL in the skin are potent producers of IL-13 upon epithelial dysregulation, which is key to the LSS response and directly regulates EC function, promotes tissue homeostasis and protects against skin carcinogenesis. This may be due to its prominent effect on skin EC, where IEL-derived IL-13 enables a canonical EC stress-response with production of IL-33 mRNA. IL-33 is an abundant cytokine 'alarmin' constitutively expressed in the nucleus of basal skin EC. Here we show that LSS regulates the level of EC IL-33 protein at steady state, as well as the release of IL-33 following topical exposure to the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA).
We also demonstrate that IL-33 protects against DMBA-induced epithelial carcinogenesis in the skin but not against subcutaneous tumour growth in a cell line-derived model. Furthermore, IL-33 restrains cellular infiltrate and epithelial hyperplasia during skin inflammation. This is possibly due to signaling via its receptor, ST2, on FoxP3$^+$ regulatory T cells (Treg), as ST2 is predominately expressed on the abundant Treg infiltrate in the DMBA-induced tumours and ST2-deficient mice are also more susceptible to inflammation-driven carcinogenesis. Together our data suggests that IL-33 is part of the LSS response to tissue dysregulation and regulates both skin immunity, EC homeostasis and carcinogenesis.

P.C6.01.19

Regulatory Innate Lymphoid Cells in tissue homeostasis and graft versus host disease

M. M. Shikhagaie, *N. Haverkade, Y. van Lier, B. Blom, M. Hazenberg;*
Amsterdam UMC, Amsterdam, Netherlands.

Background: Allogenic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment for patients with hematologic malignancies, including acute myeloid leukemia. However, 50-70% of allo-HSCT recipients develop graft-versus-host-disease (GvHD). Damage of host tissues, such as the skin and gastrointestinal tract, caused by conditioning therapy are key in the pathophysiology of GvHD since release of danger-associated molecular patterns (DAMPs) activate allo-reactive immune responses. ILCs have an essential role in tissue homeostasis and -healing, for instance in the context of allo-HSCT and GvHD, due to their location at barrier surfaces and responsiveness to cytokines produced by activated cells in their local environment. DAMPs, such as extracellular ATP (eATP),interact with ectonucleotidases that are involved in the hydrolysis of eATPinto the immunosuppressive metabolite adenosine. We studied ectonucleotidase expression on ILC and the role of eATP on ILC function.
Methods & Results: Using flow cytometry, we demonstrated that tissue-derived human ILCs, particular IL-22 producing ILC3 contributing to tissue healing, express ectonucleotidases (Ecto$^+$ILCs). Ecto$^+$ILCs hydrolysed eATP into adenosine, measured by tandem Mass-spectrometry. Genes and cytokines that regulate expression of ectonucleotidaseson tissue and peripheral blood (PB) ILC were analysed by *in vitro* studies. Moreover, altered ectonucleotidase expression was observed on ILC, but also T cells, from PB and intestinal biopsies taken from patients with GvHD when compared to controls.
Conclusion: We hypothesise that Ecto$^+$ILCs represent a novel subset of innate immune cells with immunosuppressive ability, that can control mucosal homeostasis and contribute to protective immunity during early stages of GvHD development.

P.C6.02 Innate control of inflammation and tissue repair - Part 2

P.C6.02.01

Identification of IL-17A as a predictive marker of clinical severity in pediatric ichthyosis

E. Bernaldo de Quirós[1], *R. Kennedy*[1], *V. Pérez*[1], *Á. Hernández-Martín*[2], *M. Campos*[3], *M. Pion*[1], *R. Correa-Rocha*[1];
[1]*Laboratory of Immune-regulation, Gregorio Marañón Health Research Institute, Madrid, Spain,* [2]*Pediatric Dermatology Division, Hospital Infantil Universitario Niño Jesús, Madrid, Spain,* [3]*Pediatric Dermatology Division, Hospital General Universitario Gregorio Marañón, Madrid, Spain.*

Introduction: Ichthyosis is a heterogeneous group of genetic cutaneous disorders that alter the structure of the skin and where the immune mechanisms are not well elucidated. Specific therapies have not been identified and the treatment is currently symptomatic. Our aim is to identify the immunological profile in ichthyosis pediatric patients and determine the effect of ustekinumab (a monoclonal antibody that targets IL-12/23p40) on their immune system.
Materials and methods: We carried out an exhaustive analysis of immunological cell subsets by flow cytometry from peripheral blood (<3ml). Percentages and absolute counts of up to 75 subsets were analyzed.
Results: We have evaluated four samples of a 2-year-old male patient: one before starting the treatment with ustekinumab and the three others over 4 months. We observed high values of TCD4+ IL-17A-producing cells (Th17) before treatment. The frequency of Th17 was initially reduced by 30% associated with a clinical improvement of the patient. Although neither the Th17 frequency decrease nor the clinical improvement remained, we observed reduction in the itch.
Conclusions: We tried to evaluate a possible benefit of ustekinumab and draw a parallel between the frequency of Th17 lymphocytes and the clinical outcome. Even though we observed a clear improvement of the symptoms associated to a Th17 frequency reduction during the first month, this amelioration was not sustained over time. However, we proposed the use of Th17 frequency as a diagnostic and predictive marker for severity, as well as a follow-up marker in these patients in a larger study group.

P.C6.02.02
INNATE LYMPHOID CELL SUBSETS IN THE PATHOGENESIS OF MULTIPLE SCLEROSIS

***G. C. Birch**, M. Sim, A. Harris, C. Reynolds, R. Nicholas, R. Boyton, D. Altmann;*
Imperial College London, London, United Kingdom.

Senior and corresponding authors, d.altmann@imperial.ac.uk and r.boyton@imperial.ac.uk
Multiple sclerosis (MS) is considered a disease of T cell autoimmunity. However, cellular studies and genetic association studies indicate the importance of interactions with innate immune subsets in pathogenesis[1,2]. For example, alterations in natural killer cell (NKC) phenotype or number have been correlated with MS disease.
Previous work in our laboratory identified two distinct subsets of $CD56^{dim}$ NKCs, based on differential expression of CD56, CD8, CD27 and CD57. The NKC subsets were defined as double positive (DP) $CD56^{dim}$CD8+CD57+CD27- and triple negative (TN) $CD56^{dim}$CD8-CD57-CD27-. Beta-interferon treatment was associated with similar ratios of DP/TN subsets to those seen in healthy controls. Transcriptomic analysis showed that the TN subset expressed markers indicative of innate lymphoid cells (ILC3), confirmed by FACS. Individuals with MS in acute relapse had a higher proportion of the TN compared to the DP $CD56^{dim}$ NKC subset.
Innate lymphoid cells are lineage-negative cells lacking RAG-mediated rearranged receptors that react rapidly to changes in their micro-environment[3]. Flow cytometric analysis of ILC3 cells in stable MS compared to acute relapsing MS patients and healthy controls has been carried out. Further analysis of selected populations using next generation RNA sequencing is underway to further understand the role of these cells in MS.
Funding: Medical Research Council UK, Wellcome Trust UK, MS Society UK.
References. (1)Gandhi R, *et al*. *Journal of neuroimmunology*.2010;221(1-2):7-14. (2)Parnell GP, Booth DR. *Frontiers in Immunology*. 2017;8:425. (3)Spits, H. and T. Cupedo (2012). Annual Review of Immunology **30**(1):647-675.

P.C6.02.03
IL-17E participates in the recruitment of neutrophils in psoriasiform skin inflammation

*L. Senra[1], A. Mylonas[2], J. Borowczyk-Michalowska[1], R. Stalder[1], C. Conrad[2], W. Boehncke[1,3], **N. C. Brembilla**[1];*
[1]Departement of Pathology and Immunology, University of Geneva, Geneva, Switzerland, [2]Department of Dermatology and Venereology, University Hospital of Lausanne, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, [3]Division of Dermatology and Venereology, University Hospitals of Geneva, Geneva, Switzerland.

Introduction: IL-17E (IL-25) is over-expressed in psoriatic lesional skin, and dermal macrophages represent a main target of IL-17E. We aimed at understanding the role of IL-17E in psoriasis. **Methods**: Macrophages were differentiated from peripheral blood monocytes of 5 healthy donors. Skin inflammation was provoked in BALB/c mice by tape-stripping or imiquimod application. Injection of rmIL-17E or anti-IL-17E blocking antibodies was used to study IL-17E-dependent effects *in vivo*. Skin-infiltrating cells were profiled by multi parameter-flowcytometry and immunohistochemistry, and gene transcript modifications revealed by nanostring and qPCR. **Results:** *In vitro,* IL-17E induced the production of inflammatory mediators by human M2 macrophages through NFkB, p38 and STAT3 activation. Supernatants of IL-17E-stimulated macrophages contained high levels of IL-8 and enhanced the chemotaxis of neutrophils compared to supernatants of resting macrophages. p38-activation was required for these effects. *In vivo*, intradermal injection of rmIL-17E in mice provoked a sustained inflammatory response compared to saline-injection. The resulting inflammatory infiltrate was skewed towards a preferential recruitment of neutrophils in disfavor of T cells. The neutrophil chemokine CXCL1, along with IFN type I-related genes, TNFα and amphiregulin, were abundantly expressed *in situ*. Noteworthy, IL-17E transcripts were upregulated in murine psoriasiform inflammation induced by tape-stripping and imiquimod, and neutralization of IL-17E led to significant reduction in the infiltration of neutrophils. In human lesional psoriatic skin, the number of IL-17E+ cells correlated with the number of neutrophils. **Conclusions:** Our data show that IL-17E favors the preferential recruitment of neutrophils in psoriasiform inflammation and define a novel role for IL-17E in psoriasis.

P.C6.02.04
First steps towards a human *in vitro* 3D arthritic joint model

***A. Damerau**, A. Lang, M. Pfeiffenberger, F. Buttgereit, T. Gaber;*
Charité - Universitätsmedizin Berlin, Berlin, Germany.

Our ultimate goal is to develop a valid human *in vitro* 3D joint model in order to simulate the pathogenesis of arthritis. The *in vitro* 3D joint model consists of different components including an (1) osteogenic and (2) chondrogenic part, (3) the joint space with synovial fluid and (4) the synovial membrane and will contain all involved cell types and thus enable interactions between cells by cell contacts and signaling molecules. Human bone marrow derived mesenchymal stromal cells (hMSC) are used to develop the different 3D tissue components that are characterized in detail using histological, biochemical and molecular biological methods as well as in vitro µCT and scanning electron microscopy. Results for single and co-cultivation are promising with respect to a successful colonization of hMSC on the used β-tricalcium phosphate particles (osteogenic component) and verification of the scaffold-free 3D chondrogenic component. Hyaluronic acid was applied to the osteochondral model (synovial fluid component) and was used for the cultivation of the confluent monolayer of hMSC, formed on a polycarbonate membrane (synovial membrane component).
One aspect of the inflammatory processes in the inflamed joint is simulated by applying human $CD15^+$ granulocytes in combination with cytokines distinctive of rheumatoid arthritis. Subsequently, we analyse the effect of the inflammation. By combining the different components in a standard 96 well format, we aim for a high throughput system for preclinical drug testing as well as a valid *in vitro* 3D disease model to study the immune mediated pathogenesis of arthritis using human material.

P.C6.02.05
Interaction of mesothelial CX3CL1 with monocytic CX3CR1 promotes peritoneal fibrosis

***A. Helmke**, J. Nordlohne, M. Balzer, N. Shushakova, H. Haller, S. von Vietinghoff;*
Division of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.

Introduction: Monocytes and macrophages express the chemokine receptor CX3CR1. It has been ascribed differential roles in fibrosis development. Its ligand fractalkine (CX3CL1) is expressed on endothelial cells, but has also been reported in the peritoneal cavity. Peritoneal fibrosis can develop after chronic exposure to otherwise biocompatible solution in peritoneal dialysis, a form of renal replacement therapy used by approximately 11% of patients with renal failure. It significantly limits the use of this therapy. The role of CX3CR1 and CX3CL1 in this process has not been described.
Methods: Fibrosis development and CX3CR1 expression were investigated in a murine peritoneal dialysis model in vivo and in murine and human primary cells in vitro by histology, flow cytometry, qPCR and ELISA.
Results: CX3CR1 expression increased in chronic peritoneal fibrosis in vivo and in mouse $CD11b^+$ monocytic cells in vitro during co-culture with fibroblasts. We identified the pro-fibrotic cytokine TGFβ as a mediator. CX3CR1 promoted chronic peritoneal fibrosis induced by sterile, pyrogen free dialysis solution. Mesothelial cells expressed its ligand CX3CL1, which exists as stalked membrane-bound and soluble form. Both forms were enhanced by peritoneal dialysis in vivo and by macrophage cytokines IL-1β, TNFα and TGFβ in vitro in the mesothelium more than in endothelial cells. IL-1β and TNFα significantly increased CX3CL1 and, in combination with TGFβ, TGFβ expression itself in human mesothelium. Serum CX3CL1 was elevated in patients recently started on peritoneal dialysis.
Conclusions: Our data identify CX3CR1-CX3CL1 interaction as a pro-fibrotic pathway in peritoneal fibrosis.

P.C6.02.06
Alarmin IL33/ST2 axis function during mucosa inflammation and cancer

***M. A. HERMOSO**[1], K. Dubois[1], M. De la Fuente[1], G. Landskron[1], D. Diaz-Jimenez[1], D. Simian[2], R. Quera[2];*
[1]Facultad de Medicina, Santiago, Chile, [2]Clinica Las Condes, Santiago, Chile.

The Interleukin-33 (IL33)/ST2 axis has been noted in numerous diseases, including asthma, rheumatoid arthritis, inflammatory bowel diseases and, more recently, in cancer and Alzheimer's disease. IL33, a member of the IL1 cytokine family is mainly associated with the induction of T-helper 2 (Th2) and alternative macrophage M2 immune responses through ST2 receptor (encoded by the *IL1RL1* gene). It is expressed as both a membrane-anchored receptor (ST2L) activated by IL33 and as a soluble receptor (sST2) with anti-inflammatory properties. Here we showed that during pathological conditions of the intestinal mucosa, such as in ulcerative colitis (UC) or colon cancer (CRC), levels of IL33 and sST2 are increased in tissue and the periphery. In UC, serum/mucosal ST2, and fecal calprotectin (FC) content correlated with clinical/endoscopic activity of patients, becomes a useful biomarker in the clinical practice. Also, IL33 content in left-sided CRC increases in patients with lymphatic metastasis and its tumor localization is associated with abundant desmoplasia. IL33 transcript levels from CAFs directly correlate with their capacity to induce cell migration and a mesenchymal phenotype in CRC cell lines, suggesting that IL33/ST2 mediates processes associated with invasion and interaction, specifically between CAFs and epithelial tumor cells. We also aimed to demonstrate that single-nucleotide polymorphisms (SNPs) in *IL1RL1* appear to be associated with gene expression regulation in UC patients. Finally, we propose that the IL-33/ST2 system in innate and adaptive immunity regulates cellular and molecular mechanisms, thus impacting on mucosal inflammatory disorder treatment and aiding prognosis.Fondecyt 1170648.

P.C6.02.08

Oncostatin M and the inflamed blood brain barrier: good, bad or both?

***E. Houben**, B. Broux, N. Hellings;*
Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium.

In healthy brain, leukocyte infiltration into the central nervous system (CNS) is limited by the blood-brain barrier (BBB). In multiple sclerosis (MS), this tightly regulated immune surveillance is hampered, leading to infiltration of myelin-specific T-cells into the CNS parenchyma. Oncostatin M (OSM) is produced in lesions of MS patients and we demonstrated in previous research that OSM protects against demyelination and enhances neurite outgrowth. Here, we hypothesize that OSM also has a protective role in CNS damage via the BBB. *In vitro* experiments reveal a decrease ($p<0.05$) in vascular cell adhesion protein 1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) expression (mRNA and protein) on mouse BBB endothelial cells after OSM treatment under inflammatory conditions. mRNA levels of tight and adherens junctions were not affected, although the effect on the protein level needs to be examined. To investigate the effect of OSM on the BBB *in vivo*, experimental autoimmune encephalomyelitis (EAE) was induced in OSMR KO animals. OSMR deficiency leads to a reduced disease score ($p=0.0351$, $F(1,42)=4.743$) during the acute phase. In contrast, tissue analysis revealed a persistent inflammatory environment in the chronic phase of EAE in OSMR KO mice. The *in vitro* data imply a protective effect of OSM on the BBB, while the *in vivo* data suggest both a disease promoting effect in the acute phase and a disease limiting effect in the chronic phase of EAE. Additional *in vitro* and *in vivo* experiments are necessary to reveal the true role of OSM on the inflamed BBB.

P.C6.02.09

Association of NLRP3 single nucleotide gene polymorphisms with the susceptibility to Relapsing-Remitting Multiple Sclerosis

***M. Izad**, D. Imani, A. Azimi, Z. Salehi;*
Medical Faculty, Tehran, Iran, Islamic Republic of.

NLRP3 inflammasome is a multi-protein complex that controls production of pro-inflammatory cytokines, IL-18 and IL-1β, through caspase-1 activation. These inflammatory cytokines play an important role in the development of multiple sclerosis (MS). The inflammasome NLRP3 gene variations and expression level have been suggested to affect the immune system activity. In this case-control study we determined the association of NLRP3 genetic variants and expression with MS. We analyzed four common single nucleotide polymorphisms (SNPs) of NLRP3 (rs-10754558, rs-35829419, rs-3806265, rs-4612666) in a group of 150 Iranian patients with relapsing remitting MS (RRMS) in comparison to 100 healthy controls using the TaqMan method. For analysis of NLRP3gene expression level, we studied a group of 37 RRMS patients (18 patients at relapse phase and 19 at remission phase, treated with IFN-β) in comparison to 22 healthy controls using Realtime PCR. In this study, we found that NLRP3 rs3806265 C allele and CC genotype were significantly more frequent in the RRMS patients t). While, the frequency of T allele significantly decreased in controls ($P<0.05$.The frequency of CG genotype at position rs10754558 was also significantly higher in the controls compared with patients ($P=0.03$). Moreover, expression level of the NLRP3 in patients at remission phase was significantly reduced in comparison with patients at relapse phase and also healthy controls ($P=0.01$ and $P=0.04$, respectively). The association of NLRP3 polymorphisms with the susceptibility of MS and reduced its expression after IFN-β therapy support the idea that NLRP3 inflammasome could have a critical role in inflammatory responses in MS.

P.C6.02.10

The link between angiogenesis and osteogenesis in spondyloarthritis

***M. H. Kaaij**[1,2], J. van Hamburg[1,2], G. Kollias[3,4], D. L. Baeten[1,2], L. M. van Duivenvoorde[1,2], S. W. Tas[1,2];*
[1]Department of Experimental Immunology, AMC/University of Amsterdam, Amsterdam, Netherlands, [2]Amsterdam Rheumatology & immunology Center, AMC/University of Amsterdam, Amsterdam, Netherlands, [3]Division of Immunology, Biomedical Sciences Research Center Alexander Fleming, Vari, Greece, [4]Department of Physiology, Medical School, National and Kapodistrian University of Athens, Athens, Greece.

Background: Spondyloarthritis is characterized by inflammation, extensive angiogenesis and pathological osteogenesis. Transmembrane (tm)TNF transgenic (tg) mice that overexpress tmTNF exhibit features of spondyloarthritis, including chronic inflammation and pathological osteogenesis. tmTNF ligation to TNF receptor 2 in endothelial cells (ECs) can induce signal transduction pathways, that may promote these processes. Of note, angiogenesis and osteogenesis are coupled by EC differentiation towards a type H ($CD31^{hi}endomucin^{hi}$) phenotype. We investigated the link between pathological angiogenesis, inflammation and osteogenesis in tmTNF tg mice.
Methods: Vertebrae from 6 and 12 weeks and 8 months old tmTNF tg mice or non-tg littermates (n=18) were prepared by cutting 60 µm thick cryosections for confocal imaging.
Results: tmTNF tg mice exhibited ectopic osteogenesis which was not observed in non-tg littermates. Immunostainings showed that type H vessels are in the vicinity of the ectopic osteogenesis and $osterix^{+}$ osteoprogenitors. Furthermore, there is increased osteogenesis, type H vessel presence and a different vessel architecture within the vertebrae of tmTNF tg mice compared to non-tg littermates that progresses with age. Non-tg littermate vertebrae only have physiological osteogenesis, which is in the metaphysis and periosteum. In addition, tmTNF tg mice exhibit altered bone marrow (BM) architecture containing extensive lymphoid aggregates, which predominantly consisted of $B220^{+}$ aggregates and contain high endothelial venules.
Conclusions: tmTNF overexpression in mice leads to development of type H vessels associated with ectopic osteogenesis. In addition, extensive lymphoid aggregates develop in the BM. Current studies are aimed at identification of signaling pathways in ECs that contribute to these processes.

P.C6.02.12

Targeting EphA signaling by dasatinib inhibits intestinal inflammation

***A. Kim**, S. Shim, S. Park, J. Myung;*
Korea Institute of Radiological & Medical Science, Seoul, Korea, Republic of.

Irradiation functions a crucial role in the pathogenesis of intestinal inflammatory diseases, which regulated by vascular permeability and leukocyte infiltration. Thus, a potential therapeutic strategy for the treatment of radiation-induced inflammatory diseases would be to administer drugs that could induce the regulation of vascular barrier and leukocyte extravasation at sites of inflammation. In the present study, we demonstrate that the clinically approved cancer drug dasatinib, a tyrosine kinase inhibitor, to control inflammation. To evaluate whether irradiation affects immune cell activity, we exposed to irradiation in human or mice-derived endothelial cell and leukocyte. Irradiated HUVEC showed increased permeability, inflammatory cytokine, and adhesion to leukocyte. In addition, irradiation activates inflammatory cells including monocyte, macrophage and neutrophil in lamina propria of mice. However, HUVEC treated with dasatinib decreased inflammatory cytokine level and adhesion to leukocyte through inhibition of p-VE-cadherin and ICAM. We next investigate what molecular signaling was regulated by dasatinib. We showed that irradiation triggered Eph/ephrin signaling, inducing inflammatory effects whereas dasatinib reduced phosphorylated Eph/ephrin. Moreover, decreased Eph/ephrin signaling can inhibit inflammation-associated cytokine and adhesion molecules in HUVEC. Treatment with dasatinib inhibits irradiation-induced vessel permeability and leukocyte infiltration in vivo. Therefore, irradiation-induced damage of endothelial and leukocyte activates inflammatory response but treatment with dasatinib could useful strategy for reducing inflammation.

P.C6.02.13

Synovial osteoprogenitor phenotype in patients with rheumatoid arthritis

*K. Barbaric Starcevic[1], N. Lukac[2,3], M. Jelic[1], A. Sucur[4,3], D. Grcevic[4,3], **N. Kovacic**[2,3];*
[1]Department of Orthopaedic Surgery, University Hospital Center Zagreb, University of Zagreb School of Medicine, Zagreb, Croatia, [2]Department of Anatomy, University of Zagreb School of Medicine, Zagreb, Croatia, [3]Laboratory for Molecular Immunology, University of Zagreb School of Medicine, Zagreb, Croatia, [4]Department of Physiology and Immunology, University of Zagreb School of Medicine, Zagreb, Croatia.

Introduction: Rheumatoid arthritis (RA) is a chronic, autoimmune joint inflammation, which results in disability due to irreversible joint destruction. Current treatments can slow the progression of the disease, but are still ineffective in a number of individuals and mainly target inflammation. Since there is increasing evidence on the ability of mesenchymal cells to promote regeneration and suppress inflammation, we focused on the phenotype of non-hematopoietic progenitor populations in synovial tissue of patients with arthritis. Materials and methods: We analyzed cellular composition of synovial tissue from 9 RA patients undergoing surgery, and 6 control patients undergoing arthroscopic treatment. Cells were released by collagenase digestion and analyzed by flow cytometry after labeling with two panels: 1. CD3-FITC, CD14-PE, 7-AAD, CD11b-PECy7, CD235a-APC, CD19-APCeF780, and 2. CD140a-PE, 7-AAD, CD105-PECy7, CD45/CD31/CD235a-APC, CD200-APCeF780. Results: Synovial infiltrate in RA had higher proportions of $CD3^{+}$ and $CD19^{+}$ cells, and similar variable proportions of $CD11b^{+}$ and $CD14^{+}$ in comparison to control samples. Amongst non-hematopoietic ($CD45^{-}CD31^{-}CD235a^{-}$) cells, proportion of $CD105^{+}$ cells was significantly increased in RA patients, whereas proportion of CD200+ cells was similar to controls. Amongst $CD200^{+}$ cells, $CD200^{+}CD105^{+}$ population was more abundant, while $CD200^{+}CD105^{-}$ cells were slightly less abundant in RA samples in comparison to healthy controls. Conclusions: There are significant differences in the composition of synovial non-hematopoietic compartment between RA patients and healthy synovia. According to experimental studies, $CD200^{+}CD105^{-}$ cells are considered as earliest osteoprogenitors, and also implicated in regulation of myeloid cell accumulation and activity. Loss of these cells might favor inflammation and arthritis progression.

P.C6.02.14
Modulation of immune cell infiltration by S1PR4

***C. Ringel**, B. Bruene, A. Weigert;*
Institute of Biochemistry I, Frankfurt am Main, Germany.

Sphingosine-1-phosphate is an immune-modulating lipid that has been shown to influence the immune response through five G-protein coupled receptors. For instance, migration of T lymphocytes into the circulation depends on sphingosine-1-phosphate receptor 1 (S1PR1). The less well understood S1PR4, mainly expressed on immune cells, has been found to modulate inflammatory responses but whether it is also involved in migration of immune cells is currently discussed. There is some indication that it is involved in neutrophil homeostasis and potentially trafficking. In an Imiquimod-induced mouse model of psoriasis we observed that a knockout of S1PR4 reduced inflammation, accompanied by a reduced infiltration of neutrophils and macrophages into the psoriatic lesions. At the same time we found a reduction in pro-inflammatory cytokines and chemokines such as IL-6, CCL2, and CXCL1. Similar data were also obtained in a model of Zymosan-induced peritonitis. This suggests a role of S1PR4 in the early stages of inflammation by promoting neutrophil and macrophage trafficking via chemokine production. Using RNAScope in mouse tissues and in vitro signaling pathway analysis, we identify the cellular source of these chemokines and the pathways downstream of S1PR4 that regulate their expression. Together, our data provide a novel feature of inflammation regulation by S1P and S1P receptors.
Supportet by the Else Kröner-Fresenius-Stiftung and the Graduate School for “Translational Research Innovation - Pharma” (TRIP).

P.C6.02.15
Adipocyte function in the mesenteric fat tissue depends on the innate immune system

***F. Schmidt**, I. Freise, R. Glauben, M. Letizia, C. Weidinger, B. Siegmund;*
Charité Universitätsmedizin Berlin, Berlin, Germany.

In Crohn’s disease the mesenteric fat tissue is creeping around the inflamed intestine. Previous data indicate that adipocytes express functional receptors of the innate immune system. Thus, translocalizing bacteria might serve as trigger for this unique finding. To address this question MyD88$^{-/-}$ and adipocyte-specific MyD88 knockout (MyD88^{AdipoqCre}) mice were studied.
MyD88$^{-/-}$ but not MyD88^{AdipoqCre} mice presented with an increased mortality upon dextran sodium sulfate (DSS)-treatment. The effector response in the mesenteric fat was reduced, paralleled by an increased bacterial translocation. MyD88^{AdipoqCre} mice revealed less severe disease as indicated by lower concentrations of IL-6 and TNF in *ex vivo* colon tissue supernatants and higher expression of IL10 in the mesenteric fat.
Creeping fat is characterized by a strong cellular infiltrate. To determine phenotypic and functional differences of the mesenteric fat tissue between MyD88$^{-/-}$, MyD88^{AdipoqCre} and wildtype (WT) mice, cellular composition as well as chemoattractive function was analyzed. In health, no differences with regard to macrophage and T cell populations were observed. To assess chemoattractive function of the mesenteric fat tissue, transmigration assays were performed. WT or MyD88^{AdipoqCre} but not LPS-stimulated MyD88$^{-/-}$ fat did attract CD4^{+} T-cells. However, LPS-stimulated fat from MyD88$^{-/-}$ and MyD88^{AdipoqCre} mice attracted less monocytes than WT fat. Remarkably, the phenotype of bone marrow-derived macrophages shifted to an anti-inflammatory upon treatment with either WT or MyD88^{AdipoqCre} fat.
These data indicate that adipocytes contribute to the milieu in the mesenteric fat with regard to cellular infiltrate as well as local mediators and depend on the innate immune system.

P.C6.02.16
Increased intra-articular granzyme M and local proinflammatory cytokine release in Rheumatoid Arthritis

***L. Shan**[1], A. C. Wensink[1], L. L. van den Hoogen[1], J. Meeldijk[1], H. M. Kok[1], L. H. Jongeneel[1], M. Boes[1], M. H. Wenink[2], C. E. Hack[1], T. R. Radstake[1], J. A. van Roon[1], N. Bovenschen[1];*
[1]University Medical Center Utrecht, Utrecht, Netherlands, [2]Sint Maartenskliniek, Nijmegen, Netherlands.

Objective: Granzymes are serine proteases involved in eliminating tumor cells and virally infected cells. In addition, extracellular granzyme levels are elevated in inflammatory conditions, including several types of infection and auto-immune diseases, such as rheumatoid arthritis (RA). While GrA and GrB have been associated with RA, a role for the other three granzymes (GrH, GrK and GrM) in this disease remains unclear. Here, we investigated the presence and role of GrM and GrK in serum and synovial fluid of patients with RA, psoriatic arthritis, and osteoarthritis.
Methods: Granzyme levels were determined in serum, synovial fluid, peripheral blood mononuclear cells (PBMCs) and synovial fluid mononuclear cells (SFMCs) of RA patients and relevant control groups. In addition, the link between GrM and other inflammatory cytokines in synovial fluid was investigated.
Results: Serum GrM and GrK levels were not affected in RA. GrM, but not GrK, levels were elevated in synovial fluid of RA patients. GrM was mainly expressed by cytotoxic lymphocytes in SFMCs with a similar expression pattern as compared with PBMCs. Intra-articular GrM expression correlated with IL-25, IL-29, XCL1, and TNFα levels. Intriguingly, purified GrM triggered the release of IL-29 from human fibroblasts *in vitro*.
Conclusions: These data indicate that GrM levels are increased in RA synovial fluid and that GrM can stimulate proinflammatory IL-29 release from fibroblasts, suggesting a role of GrM in the pathogenesis of RA.

P.C6.02.17
The anti-microbial peptide LL-37 shapes the response of keratinocytes to psoriasis related stimulations

***H. Sigurgrímsdóttir**[1,2], J. Freysdottir[1,3,2], B. R. Lúðvíksson[1,2];*
[1]Immunology department, Landspitali - The National University Hospital of Iceland, Reykjavík, Iceland, [2]Faculty of Medicine, Biomedical Center, University of Iceland, Reykjavík, Iceland, [3]Center for Rheumatology Research, Landspitali – The National University Hospital of Iceland, Reykjavík, Iceland.

Introduction: Psoriasis is a common inflammatory disease of the skin. It is characterized by infiltration of immune cells into the skin, hyperproliferation of keratinocytes in the basal layer of the epidermis and an imbalanced cytokine and chemokine environment. LL-37 is an anti-microbial peptide that is a part of the innate immune system. It is upregulated in psoriatic patients, both in blood and skin. The main goal of this research was to map out the effects that LL-37 has on the secretion of keratinocytes. Material and methods: A HaCaT keratinocyte cell line and primary keratinocytes were cultured and stimulated with psoriasis related stimulations or left unstimulated, all in the presence or absence of LL-37. Cell supernatant was collected and analysed with Luminex for 27 soluble analytes. Results: All chemokines, cytokines and growth factors analysed were measureable in one or more of the culture conditions. LL-37 had measurable effects on the secretion of both immortalized and primary keratinocytes. LL-37 had diverse effects and was able to decrease secretion of some analytes and increase secretion of other analytes. Keratinocytes were shown to produce IL-17A but the addition of LL-37 did not affect the secretion. Other analytes affected included IL-10, IL-17E, IL-12p70, CXCL8, CCL11, CCL19 and VEGF. Conclusions: Keratinocytes secrete many types of chemokines, cytokines and growth factors and LL-37 has a considerable effect on the secretion. Keratinocytes seem to have a bigger role in the pathogenesis of psoriasis than previously thought and that role seems to be, in part, shaped by LL-37.

P.C6.02.18
Au nanorod-induced NLRP3 inflammasome activation is mediated by ER stress

***R. J. Vandebriel**[1], S. Remy[2], J. Vermeulen[1], E. Hurkmans[1], N. Bastus[3], B. Pelaz[4], V. Puntes[3], W. Parak[4], J. Pennings[1], I. Nelissen[2];*
[1]National Institute for Public Health and the Environment, Bilthoven, Netherlands, [2]VITO NV, Mol, Belgium, [3]Institut Catala de Nanociencia i Nanotecnologia (ICN2), Barcelona, Spain, [4]Philipps Universitat, Marburg, Germany.

The widespread and increasing use of engineered nanomaterials (ENM) increases the risk of human exposure, generating concern that ENM may provoke adverse health effects. In this respect, their physicochemical characteristics are critical. The immune system may respond to specific ENM properties by inflammatory reactions. Inflammasome activation has drawn significant attention since inflammasomes, especially NLRP3 respond to a wide range of stimuli including nanoparticles, and their activation is associated with various inflammatory diseases, including lung fibrosis, obesity and type-2 diabetes. Inflammasomes are intracellular multiprotein complexes that assemble upon stimulation, resulting in activation of caspase-1 that in turn induces production of IL-1β and IL-18, which are potent mediators of inflammation. Endoplasmic reticulum (ER) stress has been reported as one of the mechanisms underlying NLRP3 inflammasome activation.
In this study, PEGylated Au ENM of ≈60 nm, having different shapes (stars, spheres and rods) were extensively characterized, and tested for possible LPS contamination. PMA-activated THP-1 cells were exposed to these ENM, and cell viability and IL-1β production were measured to assess NLRP3 activation. In addition, the exposed cells were subjected to gene expression analysis using microarray analysis to investigate related signalling pathway regulation.
PEGylated Au nanorods (NR), but not nanostars or nanospheres, showed NLRP3 inflammasome activation. Cells deficient in the NLRP3 scaffold or ASC adaptor did not show this effect. Only NR-exposed cells showed down-regulation of ER-associated cholesterol metabolism.
This may suggest that ER stress mediates NLRP3 inflammasome activation by Au NR.
Supported by the EU funded project FutureNanoNeeds (Grant agreement N° 604602).

P.C6.02.19

Imiquimod inhibits mitochondrial Complex I for K^+ efflux-independent NLRP3 inflammasome activation and cancer cell growth arrest

*D. Dittlein[1], C. J. Groß[2], J. Smollich[2], D. Stößel[3], E. Neuwirt[4], O. Gorka[4], C. Traidl-Hoffmann[1], **O. Groß**[4];*
[1]Institute of Environmental Medicine, Helmholtz Center Munich, Munich, Germany, [2]Institute of Clinical Chemistry and Pathobiochemistry, Klinikum rechts der Isar, Munich, Germany, [3]Metabolomic Discoveries GmbH, Potsdam, Germany, [4]Institute of Neuropathology, Freiburg, Germany.

Imiquimod is a small-molecule ligand of TLR7 licensed for topical treatment of skin cancers. It has TLR7-independent activities that are mechanistically unexplained, including NLRP3 inflammasome activation and induction of cancer cell growth arrest. We reported that imiquimod triggers K^+ efflux-independent NLRP3 activation in myeloid cells by inferring with cellular respiration and triggering mitochondrial ROS (Groß CJ *et al., Immunity,* 2016). Using chemoproteomics, metabolomics, and functional assays, we find that imiquimod directly inhibits mitochondrial respiratory chain Complex I, causing vast changes in cancer cell metabolism. Complex I regenerates NAD^+ required for oxidizing nutrients to support the biosynthetic pathways fueling cancer cell growth. Indeed, restoring NAD^+ regeneration by bypassing Complex I rescues proliferation. Together, our data suggest that inhibition of Complex I by imiquimod contributes to its growth-suppressive therapeutic effects on cancer, and that tissue targeting through topical application avoids systemic toxicity of respiratory chain inhibitors and is likely involved in the clinical success of imiquimod.

P.C6.02.20

Context-specific regulation of monocyte surface IL7 expression and soluble receptor secretion by a common autoimmune risk allele

***H. Al-Mossawi**, C. Taylor, N. Yager, E. Lau, S. Danielli, J. De Wit, J. Gilchrist, I. Nassiri, E. A. Mahe, W. Lee, L. Rizvi, S. Makino, J. Cheeseman, M. Neville, J. C. Knight, P. Bowness, B. P. Fairfax;*
University of Oxford, Oxford, United Kingdom.

IL-7 is a key factor in T-cell immunity and *IL7R* polymorphisms are implicated in autoimmune pathogenesis. IL7R mRNA is induced in stimulated monocytes in a genetically determined manner, yet a role for IL7R in monocyte biology remains unexplored. Here we characterize genetic regulation of IL7R at the protein level across multiple cell subsets and conditions in healthy individuals. We find monocyte surface and soluble IL7R (sIL7R) protein is markedly expressed in response to lipopolysaccharide (LPS). We further demonstrate alleles of rs6897932, a non-synonymous IL7R polymorphism associated with susceptibility to Multiple Sclerosis, Ankylosing Spondylitis and Primary Biliary Cirrhosis, form the key determinant of both surface IL7R and sIL7R in the context of inflammation. No effect of this allele was observed in unstimulated monocytes or in other lymphoid cells. Monocyte-derived sIL7R was greatly in excess of that produced by $CD4^+$T-cells, and strongly associated with both rs6897932 genotype and expression of the splicing factor gene DDX39A. Stimulated monocytes are sensitive to exogenous IL-7, which elicits a defined transcriptional signature. Flow cytometry and single cell sequencing of synovial fluid derived monocytes from patients with Spondyloarthritis shows an enlarged subset of IL7R+ monocytes with a unique transcriptional profile that markedly overlaps the in-vitro IL-7 induced geneset and the previously described mono4 subset. These data demonstrate disease-associated genetic variants at *IL7R* specifically impact monocyte protein IL7R and sIL7R following innate immune stimulation, suggesting a previously unappreciated key role for monocytes in IL-7 pathway biology and IL7R-associated diseases.

P.C6.03 Innate control of inflammation and tissue repair - Part 3

P.C6.03.01

Gastrointestinal helminth infection improved insulin sensitivity and decreased systemic inflammation in mouse models of T2D

***Z. Agha**, R. Alhallaf, M. Field, A. Kupz, J. Sotillo, A. Loukas;*
AITHM, Cairns, Australia.

The incidence of type 2 diabetes (T2D) is rapidly increasing worldwide and is becoming a major health problem particularly in western countries. Microorganisms, including parasitic worms and gut microbiota, have co-evolved with their host over millennia. It has been suggested that helminth infections might protect against T2D by inducing a T-helper-2 polarization of the immune system. On the other hand, recent data suggest that many inflammatory diseases, and in particular T2D, might be associated with gut microbiota alternations. Moreover, other studies have highlighted the role of helminth infections in altering the composition of the gut microbiota.
We here demonstrated that infection with the parasitic nematode *Nippostrongylus brasiliensis* significantly reduced body weight, fasting blood glucose and oral glucose tolerance test in mouse models of T2D. We also found that this infection was associated with boosted type 2 immune responses measured by an increase in the eosinophil number. We further investigated the effect of this helminth infection on the gut microbiota composition. Interestingly, we found that *N. brasiliensis* infection altered the gut microbiota composition, resulting in a general increase of bacteria belonging to phyla Firmicutes, Bacteroidetes and Actinobacteria, in particular orders; Clostridiales, Bacteroidales and Coriobacterials.
Our findings show that *N. brasiliensis* infection is associated with changes in the gut microbiota and in local and systemic cell populations. These changes might restore gut homeostasis and improve systemic inflammation, suggesting that this helminth might be a novel therapeutic approach for preventing T2D.

P.C6.03.02

P2X7receptor stimulation could differentially induce activation or inhibition of theNLRP3 inflammasome

***D. Angosto Bazarra**, J. J. Martínez García, H. Martínez Banaclocha, C. de Torre Minguela, P. Pelegrín Vivancos;*
Instituto Murciano de Investigación Biosanitaria IMIB-Arrixaca, Murcia, Spain.

Inflammasomes are important signaling complexes formed by a subgroup of intracellular pattern recognition receptors that activate caspase-1 and promotes the release of the proinflammatory cytokines as interleukin (IL)-1b. Among all inflammasomes the NLRP3 could be activated in response to different microbial- or damage-associated molecules, as high extracellular concentrations of the nucleotide ATP that activates the P2X purinoceptor 7 (P2X7). The NLRP3 inflammasome signals through aggregation ASC into large particle-like structures, termed ASC-specks, that could be found in biological fluids upon inflammasome activation. Our results show that stimulation of the P2X7 receptor before LPS priming and NLRP3 activation decreased the formation of intracellular ASC specks and impaired the release of IL-1b. NLRP3 priming was decreased when P2X7 was activated before LPS stimulation by studying gene expression for *Nlrp3* and *Il1b*. Treatment of the cells with the antioxidant pyrrolidine dithiocarbamate (PDTC) during initial P2X7 receptor stimulation restored IL-1b release induced by NLRP3 activation, suggesting that P2X7 receptor induced NLRP3 inhibition could be mediated by mitochondrial dysfunction. P2X7 receptor activation leads to a fast mitochondrial depolarization. To support this idea, NLRP3 activation was also reduced when mitochondrial membrane depolarization was induced by antimycin A. Similarly, mitochondrial membrane depolarization induced by antimycin A resulted in a reduction of *Nlrp3 and Il1b* gene induction by LPS. In conclusion, our data supports a novel model of NLRP3 inhibition induced by P2X7 receptor affecting mitochondrial membrane potential that affected LPS priming.

P.C6.03.03

Involvement of VEGF -1154A/G and VEGF -2758A/C in genetic susceptibility to develop RA among Algerian patients

***M. Benidir**[1], S. S. Salah[1], M. Djennane[2], H. Boulares[1], D. Zaoui[1], A. Hamdi[1], F. Mechid[3], D. Acheli[4], A. Moussa Mebarek[4], H. Balaouane[1], C. Dahou-Makhloufi[3], H. Djoudi[4], H. Amroun[5], R. Tamouza[6], N. Attal[1];*
[1]Immunology. Pasteur Institute of Algeria, Algiers, Algeria, [2]Rheumatology. Tizi-Ouzou university hospital, Tizi-Ouzou, Algeria, [3]Rheumatology. Bab El Oued university hospital, Algiers, Algeria, [4]Rheumatology. Douera university hospital, Algiers, Algeria, [5]Immunology. Hussein Dey university hospital, Algiers, Algeria, [6]Immunology and Immunogenetics. Saint Louis hospital, Paris, France.

Introduction: Inflammatory process, in RA, leads to joints destruction and can reach lungs, skin and other organs. This is important in understanding genetic background as well as role of environment in this condition. In addition, overlap of risk alleles in RA with those of other autoimmune diseases suggests existence of common mechanisms underlying these conditions. The aim of our study is to analyze possible contribution of VEGF SNPs in RA susceptibility among Algerian patients.
Methods: Our study included 228 RA patients (age: 47±13 years; Sex Ratio: 1:6; Disease duration: 9.4±8.2 years) compared to 188 healthy controls (age: 35±10 years; Sex Ratio: 1:2). Genomic DNA from RA patients and controls was genotyped, using TaqMAN technology, for VEGF-2578A/C, -1154A/G and -634C/G SNPs. Allele and genotype frequencies were compared using chi-square test. We used Phase 2.1 software to generate haplotypes.
Results: Allelic frequency analysis of VEGF-2578A/C SNP shows that allele C is more common in RA patients (OR=1.5) and that allele A is more common in healthy controls (OR = 0.67), CC genotype is more common in RA patients (OR=1.91) and AC is more common in healthy controls (OR=0.51). For VEGF-1154A/G, allele G is more common in RA patients (OR=1.53) and allele A is more common in healthy subjects (OR=0.65). In addition, GG genotype is more common in RA patients (OR=1.9) and AG genotype more common in healthy controls (OR=0.47). Finally, our results are in agreement with literature data.
Conclusion: Our results show a combination of VEGF-1154A/G and VEGF-2758A/C SNPs in RA development.

P.C6.03.04

RNA and histones in dead cells synergistically provoke FSAP activation in serum

I. Bulder[1]*, G. Marsman*[1]*, F. Stephan*[1]*, B. Luken*[1]*, S. Zeerleder*[1,2]*;*
[1]*Sanquin Research, Dept of Immunopathology and Landsteiner Laboratory, Academical Medical Center, University of Amsterdam, Amsterdam, Netherlands,* [2]*Department of Hematology, Acedemical Medical Center, Amsterdam, Netherlands.*

The plasma serine protease Factor VII-activating protease (FSAP) has been implicated in thrombosis and vascular remodeling. Moreover, FSAP is crucially involved in the release of chromatin from dead cells. FSAP circulates in an inactive form and is activated upon contact with late apoptotic or necrotic cells. FSAP binds to purified negatively charged molecules, e.g. RNA and DNA, and positively charged molecules, e.g. histones, which have both been implicated in the auto-activation of FSAP albeit through different mechanisms. We aimed to identify the component(s) from dead cells that mediate FSAP auto-activation in serum.Binding of FSAP to late apoptotic Jurkat cells was studied using (confocal) microscopy and flow cytometry. Digestion of RNA in late apoptotic cells markedly reduced the binding of FSAP to these cells, and concurrently the activation of FSAP induced by these cells was also reduced. In contrast, DNA digestion strongly enhanced both the binding and activation of FSAP. Upon cellular fractionation, the cytoplasmic fraction containing most RNA did not induce FSAP activation, whilst the nuclear fractions that predominantly contained histones did induce the activation of FSAP. In serum, the addition of histones induced FSAP activation, whilst activation did not occur upon addition of RNA. However, when RNA was combined with histones this markedly enhanced the activation of endogenous FSAP in serum. Our results indicate that both RNA and histones are involved in the activation of FSAP by dead cells. RNA does not directly induce the auto-activation of FSAP, but may promote/facilitate auto-activation of FSAP induced by histones.

P.C6.03.05

An immunosuppressive tick salivary gland protein DsCystatin interferes with Toll-like receptor signaling by downregulating TRAF6

J. Dai*;*
Institute of Biology and Medical Sciences, Soochow University, Suzhou, China.

Ticks, blood-feeding arthropods, secrete immunosuppressive molecules that inhibit host immune responses and provide survival advantages to pathogens. In this study, we characterized the immunosuppressive function of a novel tick salivary protein, DsCystatin, from *Dermacentor silvarum* of China. DsCystatin directly interacts with human Cathepsins L and B and inhibited their enzymatic activities. DsCystatin impaired the expression of inflammatory cytokines such as IL1β, IFNγ, TNFα and IL6 from mouse bone marrow derived macrophages (BMDMs) that stimulated with LPS or *Borrelia burgdorferi.* Consistently, DsCystatin inhibited the activation of mouse BMDMs and bone marrow derived dendritic cells (BMDCs) by downregulating the surface expression of CD80 and CD86. Mechanically, DsCystatin inhibited LPS or *B. burgdorferi* induced NFκB activation. For the first time, we identified that DsCystatin attenuated TLR4 signaling by targeting TRAF6. DsCystatin enhanced LPS induced autophagy, mediated TRAF6 degradation *via* an autophagy dependent manner, thereby impeded the downstream phosphorylation of IκBα and the nuclear transport of NFκB. Finally, DsCystatin relieved the joint inflammation in *B. burgdorferi* or Complete Freund's adjuvant induced mouse arthritis models. These data suggest that DsCystatin is a novel immunosuppressive protein and can be potentially used in the treatment of inflammatory diseases.

P.C6.03.06

Signaling through purinergic receptor P2Y2R enhances macrophage IL-1beta production

G. de la Rosa*, A. Gómez, P. Pelegrín;*
IMIB-Arrixaca, El Palmar, Murcia, Spain.

Release of nucleotides during processes such as necrosis or apoptosis have been described to have both proinflammatory and antiinflammatory effect on the surrounding cells. Purinergic receptors expressed sentinel macrophages will be able to modulate the inflammatory response depending on the context. Here we describe how UTP and ATP enhance IL-1beta production during Lipopolysaccharide(LPS)-induced murine resident peritoneal macrophage activation. Inhibition of the purinergic receptor P2Y2R with AR-C 118925xx reverted this increase induced by nucleotides. Moreover, blockade of Jnk activity with SP600125 also showed a reduction in IL-1beta by nucleotide-treated macrophages, indicating that this kinase is downstream P2Y2R and involved in the synergistic effect of nucleotide signaling with LPS. However, production of another proinflammatory cytokine such as TNF-**α** was clearly decreased, as per activation of adenosine receptors. This novel unique proinflammatory signature might be relevant in future inflammation studies.

P.C6.03.07

C-reactive protein promotes inflammation through metabolic reprogramming of human macrophages

M. Newling[1]*, L. Sritharan*[1]*, A. van der Ham*[2]*, M. van Weeghel*[1]*, L. de Boer*[1]*, S. Zaat*[1]*, D. Baeten*[1]*, R. Houtkooper*[1]*, B. Everts*[2]*,* ***J. den Dunnen***[1]*;*
[1]*AMC, Amsterdam, Netherlands,* [2]*LUMC, Leiden, Netherlands.*

C-reactive protein (CRP) is an acute-phase protein produced in high quantities by the liver in response to infection and during chronic inflammatory disorders, and is therefore in widespread clinical use as a marker of inflammation. Although CRP is known to facilitate the uptake of dead cells and particular strains of bacteria by phagocytic cells, it still remains largely elusive whether CRP displays additional immunological functions.
Strikingly, we here provide evidence that CRP is not only a marker, but also a cause of inflammation by strongly amplifying pro-inflammatory cytokine production. We show that complexed CRP (c-CRP), as a result of binding to its ligand phosphocholine on dead cells or bacteria, but not soluble CRP, strongly enhances TNF, IL-1β, and IL-23 production by human macrophages. While c-CRP does not induce cytokine production individually, c-CRP synergizes with particular pattern recognition receptors such as Toll-like receptors (TLRs) to amplify cytokine gene translation.
By screening with specific blocking antibodies we identified Fc gamma receptor I and IIa (FcγRI and FcγRIIa) as the main receptors responsible for initiating c-CRP-induced inflammation. Furthermore, we demonstrate that the increased production of pro-inflammatory cytokines was dependent on signaling through kinases Syk, PI3K, and Akt, resulting in enhanced pro-inflammatory cytokine production through metabolic reprogramming, particularly through amplified glycolysis, amplified fatty acid synthesis, and strongly reduced oxidative phosphorylation.
These data indicate that c-CRP-induced metabolic reprogramming provides a novel mechanism of host defense against bacteria, but may also exacerbate pathology in the context of various CRP-associated inflammatory disorders, including rheumatoid arthritis and atherosclerosis.

P.C6.03.08

Potential beneficial effects of Poly (ADP-ribose) polymerase-1 inhibition in COPD pathogenesis

V. Dharwal*, A. S. Naura;*
Department of Biochemistry, Panjab University, Chandigarh, India.

Chronic obstructive pulmonary disease (COPD) is one of the leading causes responsible for global morbidity and mortality, and has no effective treatment available till date. We have previously reported that PARP-1 plays a crucial role in the establishment of airway inflammation associated with asthma/acute lung injury. In the present work, we have evaluated the beneficial effects of PARP-1 inhibition on COPD pathogenesis utilizing elastase-induced mouse model of the disease. Our data show that PARP-1 inhibition by olaparib (5mg/kg b.wt (*i.p.*)) significantly reduced the elastase induced recruitment of inflammatory cells particularly neutrophils. Reduction in the lung inflammation was associated with suppressed myeloperoxidase activity and restoration of redox status in the lung tissues towards normal. Further, the normalization of redox status in lungs was coupled with suppressed PARP-1 activity as reflected by reduced ribosylation of tissue proteins. Western-blot analysis showed that olaparib administration prior to elastase instillation blunted the phosphorylation of P65-NF-κB at Ser 536 without altering the phosphorylation of its inhibitor IκBα in the lungs. The elastase-induced expression of NF-κB dependent pro-inflammatory cytokines (TNF-α, IL-6), chemokine (MIP-2), and growth factor (GCSF) was down-regulated severely both at the mRNA and protein levels upon olaparib administration. A significant protection against the elastase-induced emphysema was also observed. Additionally, it was observed that PARP-1 heterozygosity has similar effects as exhibited by olaparib on elastase-induced inflammation and emphysema. Overall, our data strongly suggest that PARP-1 plays a critical role in elastase induced lung inflammation, and thus might act as a potential drug target against COPD.

P.C6.03.09

ATF4 and XBP1 regulate the expression of ULBP1 in kidney cells upon endoplasmic reticulum stress activation

P. Díaz Bulnes[1]*, A. S. Sanz Bartolome*[2]*, A. Baragaño Raneros*[1]*, R. M. Rodriguez*[1]*, C. López Larrea*[1]*, B. Suarez-Alvarez*[1]*;*
[1]*Traslational Immunology Laboratory, Instituto de Investigación Sanitaria del Principado de Asturias, Oviedo, Spain,* [2]*Laboratory of Nephrology, IIS-Fundacion Jimenez Diaz, Madrid, Spain.*

Acute kidney injury may result from a variety of processes such as hypoxia, inflammation and proteinuria that lead to development and progression of kidney disease. As consequence, dysfunction of Endoplasmic Reticulum (ER) is triggered causing the activation of unfolded protein response (UPR). Expression of NKG2D ligands in damaged kidneys can induce the activation of cytotoxic cells (NK and $CD8^+$ T) through the interaction with NKG2D receptor. Our aim was to analyse the influence of ER stress on the NKG2DL expression during the induced kidney damage. Activation of UPR by tunicamycin and thapsigargin in human and mouse renal tubular cell lines induces the expression of protein sensors (ATF6, PERK and IRE1α) that initiate the three major signalling pathways of UPR. Moreover, that correlates with a significant increased of human ULBP1 ligand and its murine homologous MULT1. Similar results were observed in experimental renal damage models (AKI /UUO) where UPR activation is associated with an increased MULT1 expression.

Activation with specific inducers of each signalling pathway and gene silencing assays showed that PERK (ATF4) and IRE1α (XBP1) are directly involved in the ULBP1 and MULT1 expression. Additionally, PERK-ATF4 signalling positively regulates the IRE1α expression increasing the *XBP1* mRNA splicing. Chromatin immunoprecipitation assays revealed that both transcription factors, ATF4 and XBP1s, bind to the ULBP1 promoter regulating its expression in renal cells. Thus, blockage of the UPR signalling pathway through PERK-ATF4 and IRE1α-XBP1 could inhibit the immune recognition of renal tubular cells mediated by the NKG2D-NKG2D ligands engagement diminishing the renal damage.

P.C6.03.10

Epidermal growth factor and mannan binding lectin in serum and saliva of patients with recurrent aphthous stomatitis

Z. Jiraskova Zakostelska[1], *Z. Stehlikova*[1], *K. Klimesova*[1], *M. Kverka*[1,2], *J. Bartova*[3], *A. Fassmann*[4], *L. Izakovicova-Holla*[4], *J. Petanova*[3], *H. Tlaskalova-Hogenova*[1];
[1]*Institute of Microbiology of the CAS, Prague, Czech Republic,* [2]*Institute of Experimental Medicine of the CAS, Prague, Czech Republic,* [3]*School of Dental Medicine, General University Hospital in Prague, Prague, Czech Republic,* [4]*Clinic of Stomatology, Institutions Shared with St. Anne's Faculty Hospital, Masaryk University, Brno, Czech Republic.*

Introduction: Recurrent aphthous stomatitis (RAS) is a multifactorial disease with unknown etiopathogenesis. Genetic and immunological background of individual together with environmental factors plays an important role in onset, progression and remission of this disease. The aim of our study was to measure changes in epidermal growth factor (EGF), a cytoprotective factor against injuries, and mannan binding lectin (MBL), a pattern recognition molecule of the innate immune system that activates lectin pathway, in saliva and serum of patients with RAS. **Methods:** Whole saliva and serum samples were collected from healthy controls (n= 15) and RAS patients in active (with oral ulceration; n= 33) or inactive (absence of oral ulceration; n= 28) stage of disease. Measurement of EGF or MBL concentration was performed by ELISA. **Results:** The levels of EGF in serum from patients in active and passive stage of RAS were significantly decreased when compared to healthy controls. In saliva, we found similar trend for EGF when we compared patients with active and passive stage of RAS and healthy controls but those result were not statistically significant. We have not found any significant differences in levels of MBL in RAS patients compared to healthy controls. **Conclusions:** EFG is one of the essential factors in tissue regeneration and its low concentration in serum or saliva may hinder healing or regeneration in patient with recurrent aphthous stomatitis. This study was supported by Ministry of Health of the Czech Republic, grant nr. 15-29336A.

P.C6.03.11

SIRPá signaling inhibits macrophage polarization towards the proinflammatory phenotype while promoting the anti-inflammatory polarization through biased activation of SHP-1 or SHP-2

Y. Liu*;*
Georgia State University, Atlanta, United States.

Macrophages undergo polarization into the proinflammatory M1 phenotype, which is required for host defense, or the alternative M2 phenotype, which is involved in wound healing and tissue remodeling. SIRPα is an Immunoreceptor Tyrosine-based Inhibitory Motif (ITIM)-containing receptor expressed on macrophages and functions through the extracellular interaction with CD47, the broadly expressed cellular ligand, which triggers SIRPα ITIMs phosphorylation leading to activation of the SH2-containing tyrosine phosphatases, SHP-1 and SHP-2. Here we examined the role of SIRPα in regulating macrophage phenotypic polarization. With SIRPα extracellular ligation by CD47 (mCD47-AP), macrophages display a defective response to M1 phenotypic activation but an enhanced reaction following the M2-polarized induction, as indicated by reduced expressions of the M1-related markers CD86, MHC-II, iNOS, IL-1β, IL-12 and TNF-α under LPS/IFNγ treatment but increased expressions of anti-inflammatory mediators CD206, Arg1, IL-10 and TGFβ under IL-4 treatment. Consistently, macrophages deficient of SIRPα obtained from $SIRP\alpha^{-/-}$ mice demonstrate enhanced M1 activities under proinflammatory challenges while showing repressed M2-associated, tissue supporting function. Interestingly, although both polarized activations induce SIRPα ITIMs phosphorylation, differential associations with SHP-1 and SHP-2 are exhibited with M1 and M2 respectively, leading to distinctive activations of the PI3k-Akt pathway, the Erk1/2 MAP kinase and IL-4-induced Jak-STAT6. Overall, our findings demonstrate for the first time that SIRPα plays an important role in modulating macrophage polarized activation, inhibiting M1 while promoting M2 phenotypic polarization through biased activation of SHP-1 and SHP-2 signaling.

P.C6.03.12

Priming of macrophages with air particulate matter SRM 1648a induces hyperinflammatory response upon LPS challenge

A. Gawda, ***G. Majka****, B. Nowak, M. Śróttek, M. Walczewska, J. Marcinkiewicz;*
Chair of Immunology, Jagiellonian University Medical College, Kraków, Poland, Kraków, Poland.

Introduction: Exposure to air particulate matter (PM) has been implicated in both chronic inflammatory as well as autoimmune diseases. Macrophages are responsible for the regulation of chronic inflammation. However, whether PM affects macrophage polarization remains unclear. The aim of this study was to evaluate if urban PM at nontoxic concentrations is able to prime macrophages to altered inflammatory response upon LPS challenge.
Methods: Two forms of the urban particulate matter SRM 1648a were used: intact PM and PM-deprived of organic compounds (PMΔC). Peritoneal murine macrophages were incubated with different concentrations of PM samples for 24h and then challenged with LPS. Production of inflammatory mediators by macrophages was measured to test immunostimulatory/priming capacity of PM.
Results: PM at non-cytotoxic concentrations induced a dose-dependent production of proinflammatory cytokines (TNF-α, IL-6, IL-12p40). By contrast, PMΔC were not able to stimulate macrophages. However, macrophages primed with both forms of PM show proinflammatory response upon LPS challenge. The ratio of proinflammatory to anti-inflammatory cytokines (TNF-α to IL-10) was significantly increased upon incubation with urban particulate matter.
Conclusion: Our data demonstrate that exposure of macrophages to low non-stimulating concentrations of PM may prime the cells to hyperinflammatory response upon contact with LPS. It is possible that exposure of patients suffering from chronic inflammatory diseases to particulate matter may be responsible for the aggravation of clinical symptoms during bacterial infections. This work was financed by the National Science Centre (Poland, project number 2015/16/W/ST5/00005).

P.C6.03.13

Identification of rare variants in genes of the inflammasome pathway in Behçet disease patients using next generation sequencing techniques

S. Burillo Sanz[1], ***I. Olivas Martinez***[1], *M. Montes Cano*[1], *J. Garcia Lozano*[1], *L. Ortiz Fernandez*[1,2], *M. Gonzalez Escribano*[1];
[1]*Hospital Virgen del Rocio, Sevilla, Spain,* [2]*Institute of Parasitology and Biomedicine "Lopez Neyra", Granada, Spain.*

Behçet's disease (BD) is an uncommon immune-mediated systemic disorder with a well-established association with HLA class I and other genes. BD has shown to have clinical overlap with many autoinflammatory diseases (AIDs). Thereby, the aim of the study was to investigate the role in BD of rare variants in seven genes involved in AIDs: CECR1, MEFV, MVK, NLRP3, NOD2, PSTPIP1 and TNFRSF1A by using a next generation sequencing (NGS) approach in 355 BD patients. To check global association of each gene with disease, 4 tests: SKAT, CollapseBt, C(α) and weighted KBAC were used. Databases: 1000 Genomes Project Phase 3, Infevers, HGMD and ClinVar and algorithms: PolyPhen2 and SIFT were consulted to collect information of the 62 variants found. All the genes resulted associated using SKAT, but only 3 (MVK, NOD2 and PSTPIP1) with C(α) and weighted KBAC. When all the genes are considered, 40 variants were associated to AIDs in clinical databases and 25 were predicted as pathogenic at least by one of the algorithms used. Including only MVK, NOD2 and PSTPIP1, the associated to AIDs variants found in BD were 20 and the predicted as pathogenic, 12. The maxima contribution corresponds to NOD2. This study supports the influence of rare variants in genes involved in AIDs in the pathogenesis of BD.

P.C6.03.14

Inflammatory phenotype of ORS (Outer Root Sheath cells) is related to a replicative stress in Hidradenitis suppurativa.

C. Orvain[1,2], *Y. Bennasser*[3], *Y. Lin*[4], *F. Jean-Louis*[1,2], *B. Hersant*[5,6,7], *C. Hotz*[8], *P. Wolkenstein*[5,6,8], *M. Boniotto*[1,2,5], *H. Hocini*[1,2], *J. Lelièvre*[1,2,6], *P. Pasero*[4], *Y. Lévy*[1,2,6], *S. Hüe*[1,2,9];
[1]*INSERM U955, Team 16, Créteil, France,* [2]*Vaccine Research Institute (VRI), Université Paris Est Créteil, Faculté de Médecine, Créteil, France,* [3]*Institut de Génétique Humaine, Laboratoire de Virologie Moléculaire, Université de Montpellier, CNRS UMR9002, Montpellier, France,* [4]*Institut de Génétique Humaine, CNRS, Université de Montpellier, Montpellier, France,* [5]*Université Paris Est Créteil, Faculté de Médecine, Créteil, France,* [6]*Assistance publique, Hôpitaux de Paris (AP-HP)-Hôpital Henri Mondor-Université Paris Est Créteil, Val-de Marne (UPEC), Créteil, France,* [7]*Plastic, Reconstructive and Aesthetic Surgery Department, Henri Mondor Hospital, Créteil, France,* [8]*Dermatology Department, Groupe Hospitalier Henri Mondor, AP-HP, Créteil, France,* [9]*Assistance Publique-Hôpitaux de Paris, Service d'Immunologie Biologique, Groupe Henri-Mondor Albert-Chenevier, Créteil, France.*

Hidradenitis suppurativa (HS) is a chronic inflammatory disease of the hair follicles (HF) leading to abscessing inflammation. We showed that Outer Root Sheath cells (ORS), HF stem cells, have a pro-inflammatory phenotype (Hotz, JID, 2016). We investigate molecular mechanisms involved in this phenotype.
Materials and Methods: ORS were isolated from skin surgery after HF dissection. RNA and protein expression were studied by RT-qPCR and Western Blot, detection of cytosolic single-stranded DNA (ssDNA) and nuclear 53BP1 foci, by immunocytochemistry, and cell cycle and HF phenotype, by flow cytometry. Replication fork progression was measured by DNA fiber spreading.
Results: The transcriptomic analysis of HS-ORS revealed (i) a type I interferon (IFN) signature confirmed by an overexpression of IFNβ and associated interferon-stimulated-genes by RT-qPCR and (ii) a deregulation of genes involved in cell proliferation and differentiation. Cytoplasmic ssDNA was observed in HS-ORS but not HS-keratinocytes. IFNβ mRNA decreased in HS-ORS transfected by siSTING compared to siControl, suggesting STING involvement in ssDNA detection. Cell cycle analysis reveals an accumulation of cells in S or G2 phase in 7 out of 11 patients, which also showed deregulated replication fork progression. CHK1 phosphorylation and 53BP1 foci observed in HS-ORS indicate an activation of DNA damage pathway. HF cells phenotype's ex-vivo analysis showed enrichment of progenitor cells compared to stem cells suggesting stem cells differentiation.
Conclusion: This suggests that in HS patients, cell cycle deregulation in the ORS leads to replicative stress and accumulation of cytoplasmic ssDNA, inducing IFN synthesis involved in establishment of chronic inflammation.

P.C6.03.15

Long term outcomes and treatment response in patients with TNF receptor-associated autoinflammatory syndrome (TRAPS): retrospective experience from a national referral centre

R. Papa[1], *D. M. Rowczenio*[2], *C. Papadopoulou*[3], *T. Rezk*[2], *P. Brogan*[3], *T. Youngstein*[2], *P. N. Hawkins*[2], *H. J. Lachmann*[2];
[1]*Autoinflammatory Diseases and Immunodeficiencies Centre, Pediatric and Rheumatology Clinic, Giannina Gaslini Institute, University of Genoa, Genoa, Italy,* [2]*National Amyloidosis Centre, Division of Medicine, Royal Free Campus, University College London, London, United Kingdom,* [3]*Department of Infection, Inflammation and Rheumatology, Great Ormond Street Institute of Child Health, London, United Kingdom.*

Introduction. Secondary, AA amyloidosis and infertility are most common and severe complications of Tumour necrosis factor Receptor-Associated Periodic Syndrome (TRAPS) in adults. **Objectives**. To define the best treatment approach in TRAPS and the effect on long-term outcomes. **Methods.** We reviewed all data of 100 patients carrying a total of 40 *TNFRSF1A* gene variants. The Auto Inflammatory Diseases Activity Index (AIDAI) was used to estimate the long-life disease severity. **Results.** 29 patients had intronic variants of the *TNFRSF1A* gene and displayed milder disease than the 71 patients with mutations affecting coding regions (P<0.005), less abdominal pain and skin rashes but more frequent headache or mouth ulcers during fever attacks (P<0.001 and P<0.05, respectively), none developed secondary amyloidosis. Almost 70% of patients required maintenance therapy and anti-interleukin (IL) 1β drugs were the most frequently used (53 patients) with the highest efficacy rate (86% complete response). No patients on anti-IL1β treatment developed amyloidosis and 10 patients with amyloidosis have been successfully treated with anti-IL1β agents with preservation of native renal function in 7 and excellent long term transplant function in 2. Seven women with a history of failure to conceive had successful pregnancies without fertility treatment following anti-IL1β therapy. **Conclusion**. Anti-IL1β drugs are the best maintenance treatment in TRAPS with potential to reverse the most serious disease complications. The diagnosis of TRAPS should be considered very carefully in patients carrying intronic variants of the *TNFRSF1A* gene. **Acknowledgments.** The presenting author thanks the European Federation of Immunological Societies for the Short-term Fellowship.

P.C6.03.16

Molecular mechanism of peptide against calcium oxalate crystals induced NLRP3-mediated IL-1β secretion

S. Saha*, R. J. Verma;*
Gujarat University, Ahmedabad, India.

Even though the prevalence of nephrolithiasis is increasing, our understanding of the underlying mechanisms has not kept pace and new therapeutic approaches have not yet emerged. Nephrolithiasis can lead to inflammation and subsequent tissue remodeling. Calcium oxalate (CaOx) is the main component of kidney stones. The present study identifies the role of a designed peptide in decreasing induction time of CaOx crystals nucleation, cytokine expression, neutrophil recruitment and subsequent renal injury. By using combination of confocal microscopy and atomic scale molecular dynamics simulations (MD), we find that FITC labeled peptide adsorb specifically with the edges of CaOx crystals. Molecular modeling of peptide onto crystal surfaces also confirms their adsorption to CaOx crystal {110} and {101} surfaces and suggests increased stabilization of the CaOx surface by peptide adsorption. Furthermore, the peptide potently inhibits the outcomes of NLRP3 inflammasome activation, including IL-1β secretion, caspase-1 cleavage, and lactate dehydrogenase release using the LPS-primed murine bone marrow-derived macrophages in the presence of ATP and/ or calcium oxalate crystals. The peptide also showed significant inhibition of the functionality of the NLRP3 inflammasome and resulting anti-inflammatory activity *in vivo* using calcium oxalate-induced nephrolithiasis in female Wistar rat model. These inhibitory effects of peptide were observed in a dose-dependent manner. These findings suggests new avenue for rational design of peptide based therapeutic agents for kidney stone diseases as well as other NLRP3 inflammasome-related diseases.

P.C6.03.17

Hyaluronic acid improve Immune modulatory effect of mesenchymal stem cells

S. Shim*, J. Lee, H. Jang, J. Myung, S. Lee, M. Kim, W. Jang, S. Lee, H. Kim, S. Park, K. Kim;*
Laboratory of Radiation Exposure and Therapeutics, National Radiation Emercency Medical Center, Seoul, Korea, Republic of.

Mesenchymal stem cells (MSCs) display a powerful immunosuppressive potential, and this potential can be employed for the treatment of inflammatory-based diseases. In this study, we investigated the cytokine expression of MSCs rely upon the expression of CD44, a putative stem cell marker and observed interesting expression changes involved in immune regulation. CD44 knock-down leads to a decreased expression of CD200, an anti-inflammatory factor, and Hyaluronic acid (HA), a ligand of CD44, increased the expression of CD200 in MSCs. Furthermore, the effect of HA-treated on MSCs was functionally determined in mouse model. HA-treated MSC demonstrated a significant reduction in inflammation as demonstrated by the decrease in the expression of pro-inflammatory markers and by the induction of a regenerative environment. Together, our results establish that CD44 pathway contributes to immunosuppressive functions of MSCs and HA treatment could improve outcomes of MSC therapies of inflammatory diseases.

P.C6.03.18

MFB, a critical regulator of inflammatory resolution, induced M1 macrophage selective apoptosis through TNF/TNFR1, modulated survival M1 conversion into M2 depended on leptin-PI3K or -Akt pathway

H. Lu, H. Zhu, R. Chen, Y. Tian, H. Xu, ***Z. Su****;*
School of Medicine, Zhenjiang, China.

Inflammatory monocytes/macrophages and tissue-resident macrophage are key regulators of tissue injury, would healing, organ remodeling and regeneration. After tissue injury, most monocytes/macrophages infiltrate into injury sites, undergo phenotypic and functional changes: differentiation into proinflammatory M1 phenotype and anti-inflammatory M2 in milieu. The polarized M1 and M2 macrophages involve in inflammatory initiation, development, resolution, and organ function remodeling, respectively. Disturbances in macrophage reprogramming can lead to aberrant repair or uncontrolled inflammatory response. Timely modulation of macrophages reprogramming is essential for the treatment of inflammatory diseases. Therefore, it becomes fatal to clarify the M1 fate or the functional shift of M1 and M2 and to explore the factor of inducing inflammatory resolution during tissue injury development. In the present work, we demonstrated that cardiac fibroblasts (MCF) pretreated by angiotensin II (ANG II) (myofibroblast, MFB) could not only promote macrophage reprogramming toward M1 but also induced M1 macrophage selective apoptosis through TNF/TNFR1 axis independent on Fas/FasL; furthermore, in co-culturing system, leptin produced by MFB or polarized macrophage could promote the survival M1 conversion into M2 macrophage via PI3K or Akt pathway. *In vivo*, TNF-α blockade significantly ameliorated experimental autoimmune myocarditis (EAM) development. Our data highlighted a novel and exciting topic: MFB is a critical and active regulator of inflammatory resolution. Our data also suggested a potential checkpoint for inflammatory disease therapy target on macrophage.

P.C6.03.19

New diagnostic markers: NLR and PLR for rheumatoid arthritis

N. Kikodze[1,2], *N. Tsiskarishvili*[3], *M. Iobadze*[1,2], *N. Janikashvili*[1], *I. Pantsulaia*[1,2], *T. Chikovani*[1];
[1]*Tbilisi State Medical University, Tbilisi, Georgia,* [2]*V.Bakhutashvili Institute of Medical Biotechnology, Tbilisi, Georgia,* [3]*Clinical Medicine Scientific-Research Institute – Todua Clinic, Tbilisi, Georgia.*

Systemic inflammation response is closely associated with many life-threatening diseases: cancer, autoimmune disorders, etc. Seeking for reliable and inexpensive markers to assess inflammation is of importance to envisage long-term outcome as well for treatment monitoring in the patients. Changes in the composition of the circulating blood cells as possible inflammatory markers are intensively investigated in the last years. We studied correlations of new inflammatory markers neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) with the known inflammatory markers: erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in the patient with rheumatoid arthritis (RA). Newly diagnosed patients both in disease active (>2.3 by DAS28 score) and in non-active (<2.3 by DAS28 score) stages have high levels of NLR and PLR compared to healthy individuals. NLR and PLR positively correlated with ESR in rheumatoid arthritis. The association between NLR, PLR, CRP and RA was not observed in our study. The correlation of between NLR, PLR and disease activity was also revealed.

P.C6.04 Innate control of inflammation and tissue repair - Part 4

P.C6.04.01

Critical contribution of NKG2D expressed on iNKT cells in Con A-induced liver hepatitis in mice

d. al dulaimi[1,2], *j. klibi*[1,2], *v. olivo pimentel*[1,2], *v. parietti*[1,3], *m. allez*[1,2], *a. toubert*[1], ***k. benlagha***[1,2];
[1]*inserm, paris, France,* [2]*1INSERM, UMR-1160, Institut Universitaire d'Hématologie, Paris, France, Université Paris Diderot, Sorbonne Paris Cité, Paris, France., Paris, France,* [3]*3Département d'Expérimentation Animale, Institut Universitaire d'Hématologie, Paris, France, Université Paris Diderot, Sorbonne Paris Cité., Paris, France.*

Natural killer group 2D (NKG2D) is a well-characterized activating receptor expressed on many immune cells including Invariant Natural Killer T (iNKT). iNKT cells were shown to be responsible of liver injury in the model of Concanavaline A (Con A)-induced hepatitis, considered to be an experimental model of human autoimmune hepatitis. In this study, we investigated whether NKG2D plays a role in the hepatitis induced by iNKT cell-mediated immune response to Con A. By using killer cell lectin-like receptor subfamily K, member 1 deficient *(Klrk1***-/-**)** mice, we found that the absence of NKG2D reduced the hepatic injury upon Con A administration. This was not due to an intrinsic functional defect of NKG2D-deficient iNKT cells. The reduced pathogenic effect of Con-A in the absence of NKG2D correlates with a reduction in pathogenic cytokine production and FAS-Ligand (FAS-L) expression by iNKT cells.

We also found that ConA administration led to an increase in the retinoic acid early-inducible 1 (RAE-1) surface expression on wild type hepatocytes. Finally, we found that Con A has no direct action on FAS-L expression or cytokine production by iNKT cells, and thus propose that NKG2D-L expression on stressed hepatocytes promote cytotoxic activity of iNKT cells via its interaction with NKG2D contributing to hepatic injury. Conclusion: our results highlight NKG2D as an essential receptor required for activation of the iNKT cells in Con A-induced hepatitis and indicate that it represents a potential drug target for prevention of autoimmune hepatitis.

P.C6.04.02
Role of neutrophils in the Imiquimod (IMQ)-induced mouse model of Psoriasis

S. Costa[1]*, D. Bevilacqua*[1]*, O. Marini*[1]*, E. Caveggion*[1]*, C. Lowell*[2]*, G. Girolomoni*[3]*, M. Cassatella*[1]*, P. Scapini*[1]*;*
[1]Department of Medicine-Section General Pathology, University of Verona, Verona, Italy, [2]University of California, San Francisco, California, United States, [3]Department of Medicine-Section of Dermatology and Venereology, University of Verona, Verona, Italy.

Psoriasis is a chronic skin disease associated with deregulated interplays between immune cells and keratinocytes. Neutrophil accumulation in the skin is one of the histological features that characterize psoriasis. However, the role of neutrophils in psoriasis development remains poorly understood. In this study, we utilized the imiquimod (IMQ)-induced mouse model of psoriasis to elucidate the specific contribution of neutrophils to psoriasis development. We report that neutrophils act as negative modulators of disease propagation and exacerbation by inhibiting γδ T cell effector functions *via* NADPH oxidase-mediated reactive oxygen species (ROS) production, as revealed by analysing disease development/progression in neutrophil-depleted mice. We also report that Syk functions as crucial molecule mediating neutrophil and γδ T cell interactions. In support of the latter findings, we demonstrate that the selective impairment of Syk-dependent signalling in neutrophils only, is sufficient to reproduce the enhancement of skin inflammation and γδ T cell infiltration observed in neutrophil-depleted mice. Overall, our findings add new insights into the specific contribution of neutrophils to disease progression in the IMQ-induced mouse model of psoriasis. Considering that, similarly to mouse psoriasis, the important role of IL-17 producing γδ T cells in human psoriasis has just started to emerge, it is likely that inhibitory crosstalk between neutrophils and γδ T cells may exist also in human psoriasis. Neutrophils may indeed act as unexpected negative players of disease development in specific types or clinical stages of human psoriasis. Consequently, also the utilization of therapeutic interventions targeted to inhibit neutrophil functions should be carefully evaluated.

P.C6.04.03
Innate-like T-lymphocyte deficiencies in the pathogenesis of chronic obstructive pulmonary disease

P. Engelmann[1]*, K. Bodó*[1]*, N. Farkas*[2]*, V. Sárosi*[3]*, Z. Balikó*[3]*, T. Berki*[1]*, M. Szabó*[3]*;*
[1]Department of Immunology and Biotechnology, Clinical Center, Medical School, University of Pécs, Pécs, Hungary, [2]Department of Bioanalysis, Medical School, University of Pécs, Pécs, Hungary, [3]Division of Pulmonology, 1st Department of Internal Medicine, Clinical Center, Medical School, University of Pécs, Pécs, Hungary.

Chronic inflammation of the small airways and the damage of lung parenchyma are considered as the major mechanisms in COPD. Recent observations claim that innate-like T-lymphocytes such as invariant natural killer T (iNKT) cells and mucosal-associated invariant T (MAIT) cells connect innate and adaptive immunity. Up to now data are scarce about their involvement in the pathogenesis of COPD. We aimed to observe the proportions of iNKT and MAIT cells in the peripheral blood and sputum samples of stable and exacerbating COPD patients. By means of multicolor flow cytometry the frequencies of total iNKT and MAIT cells and their subsets were enumerated. In addition, the expression levels of iNKT, and MAIT TCR genes, along with CD1d, MR1 genes were assessed by qPCR in the study cohorts. Proportions of total iNKT and MAIT cells were dramatically dropped in COPD blood samples. In the sputum of COPD patients reduced numbers of iNKT cells were observed. Furthermore decreased DN and increased CD4+ iNKT subsets, while elevated DN and dropped CD8+ MAIT subpopulations were measured in COPD samples. Reduced invariant TCR mRNA levels in COPD patients had confirmed these findings. CD1d and MR1 mRNA expression were increased in stable and exacerbating COPD patients. In contrast, both molecules were decreased following antibiotic and systemic steroid treatments. Our observations support the notion that iNKT and MAIT cells are involved in COPD. These innate-like T-lymphocytes deserve further analysis to validate their usefulness as potential biomarkers in the pathogenesis of this disease.

P.C6.04.04
The effects of intravenous immunoglobulin (IVIg) on peripheral blood polymorphonuclear cells

B. Geçkin*, B. Kayaoğlu, M. Gürsel;*
Department of Biological Sciences, Middle East Technical University, Ankara, Turkey.

IVIg has been used in clinical treatment of primary immune deficiencies (PIDs) and several autoimmune diseases. Neutrophil dysregulation frequently observed in a variety of PIDs improve after IVIg treatment. This study aimed to assess the effects of IVIg on neutrophil functions as well as the response of IVIg treated innate immune cells to various TLR and/or nucleic acid sensor ligands. PBMCs from healthy subjects were isolated by density gradient separation. For neutrophil isolation, polymorphonuclear cells that pelleted with the erythrocytes were further separated by dextran sedimentation. Neutrophils were stimulated with LPS, Zymosan or PMA in the absence or presence of increasing concentrations of IVIg (0.2, 1, 5, 25 mg/ml).
Reactive oxygen species (ROS) production was quantified using DHR123 staining and flow cytometry. Similarly, the effect of IVIg treatment on LPS, Zymosan or PMA-induced NETosis was assessed using nucleic acid probes and fluorescence microscopy. Additionally, response of PBMCs to various pattern recognition receptor ligands was determined in the presence of four different concentrations of IVIg by cytokine ELISA. Results revealed that response to potent ROS inducers like PMA was significantly suppressed with the highest dose of IVIg, whereas immunoglobulin treatment synergized with LPS to increase ROS production. Our data reflects that IVIg exerts either stimulatory or anti-inflammatory activity on granulocytes in a dose and stimulant-dependent manner.

P.C6.04.05
Fragile neutrophils in the peripheral blood: a new phenomenon in critically ill patients.

L. Hesselink*, R. Spijkerman, P. Hellebrekers, A. Huisman, I. E. Hoefer, M. ten Berg, L. Koenderman, L. P. Leenen, F. Hietbrink;*
UMC Utrecht, Utrecht, Netherlands.

Introduction: Non-viable (propidium iodide/PI-positive) neutrophils are incidentally found in blood samples of critically ill patients. Not much is known regarding the function of these neutrophils and the clinical implications of these neutrophils in the circulation. The aim of this study was to investigate overall neutrophil function and clinical course of patients who have non-viable neutrophils.
Methods: The percentage of PI-positive neutrophils is routinely measured in every blood analysis, as indicator for timely analysis. However, surgical ward and ICU samples are immediately analyzed. Surgical patients who had > 5% PI-positive neutrophils, were included. After inclusion, the percentage of PI-positive neutrophils was reassessed by flow analysis. In addition, phagocytosis was analyzed.
Results: Thirteen surgical patients were included. The high PI signal originated either from increased neutrophil autofluorescence (n = 7) or the presence of fragile neutrophils (n = 6). Fragile neutrophils were neutrophils that became PI-positive after minimal *ex vivo* manipulation (red blood cell lysis) or when kept in EDTA tubes. Four of 6 patients with fragile neutrophils died. In contrast, no patients with autofluorescent neutrophils died. Phagocytosis of patients with fragile neutrophils was not impaired.
Conclusion: Non-viable neutrophils detected by the hematology analyzer are either true fragile neutrophils or autofluorescent neutrophils. Fragile neutrophils are easily missed during in vitro experiments as they are typically lost during standard isolation procedures. The presence of fragile neutrophils was associated with a high mortality rate and thus renders more attention to determine their role in the clinical course of critically ill patients.

P.C6.04.06
Extracelluar neutrophil-derived glycosidases - new post-secretional modifiers of human Immunoglobulin G glycosylation?

J. Knopf*, L. E. Munoz, M. Herrmann;*
Universtaetsklinikum Erlangen - Medizin 3, Erlangen, Germany.

Glycosylation of Asparagine[297] of Immunoglobulin G (IgG) heavy chain influences the effector functions of IgG and contributes to the pathogenesis of chronic inflammatory autoimmune diseases such as Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA). We employed mass spectrometry to analyze the glycosylation of total IgG from patients with SLE or RA compared to acute inflammatory diseases such as sepsis, as well as healthy controls. Patients with sepsis showed a similar IgG glycosylation pattern as RA patients with low levels of galactose and bisecting N-acetylglucosamine. Surprisingly, the glycosylation pattern of IgG from patients with SLE displayed only minor changes when compared to healthy controls. This is most likely due to the successful treatment and the concomitant low disease activity in our SLE cohort.
We tested whether the changes observed for sepsis and RA patients are due to post-secretional modifications by neutrophil extracellular traps (NETs)-borne glycosidases of IgG glycans. The potential glycan modifiers neuraminidase1, beta-galactosidase and beta-N-acetylglucosaminidase were expressed in human neutrophils and aggregated NETs contained enzyme activities as assessed by specific substrate conversion. Strikingly, co-incubation of IgG with aggregated NETs reduced the percentage of bisecting N-acetylglucosamine.
To further analyze the role of glycosidases in modifying IgG glycans, we are going to express the specific glycosidases and analyze their effect on the glycans of circulating IgG and its functional implications *in vivo*.
221 words

P.C6.04.07

Implication of type 2 innate lymphoid cells in skin fibrosis during systemic sclerosis

P. Laurent[1], *M. Jeljeli*[2], *P. Manicki*[3], *V. Jolivel*[1], *C. Richez*[1,3], *P. Duffau*[1,4], *E. Lazaro*[1,4], *J. Déchanet-Merville*[1], *P. Blanco*[1,5], *T. Pradeu*[1], *C. Contin-Bordes*[1,5], *M. Truchetet*[1,3];
[1]*ImmunoConcept, UMR 5164,, Bordeaux, France,* [2]*Immunology laboratory, EA 1833, Paris, France,* [3]*Rheumatology department, CHU Bordeaux Hospital,, Bordeaux, France,* [4]*Internal Medicine department, CHU Bordeaux Hospital, Bordeaux, France,* [5]*Immunology department, CHU Bordeaux Hospital, Bordeaux, France.*

Systemic sclerosis (SSc) is an autoimmune disease characterized by vascular abnormalities, immune disorders and fibrosis. Recently, some reports highlighted the fundamental role of type 2 innate lymphoid cells (ILC2s) in fibrosis. We hypothesized that ILC2s could be involved in SSc fibrosis, offering new therapeutic perspectives.
Methods: We quantified circulating and cutaneous ILCs in healthy donors and SSc patients by flow cytometry and immunofluorescence. In HOCl-induced SSc mice, we phenotyped ILC2s in skin, lung and spleen.
Results: In 39 SSc patients, circulating ILCs decreased in absolute value (0.019 for HD vs 0.055 for patients, p<0.0001) compared to 18 controls, mainly in ILC2s (0.0138 for HD vs 0.00767 for SSc, p=0.0496) and ILC3s (0.02152 for HD vs 0.003839 for SSc, p<0.0001). We observed an inverse correlation between circulating ILC2s and rodnan scores in SSc patients (R=-0.03, p=0.02). A skewing toward ILC2 was also observed in SSc skin patients. HOCl-induced SSc mice showed an early increase in absolute count of ILC2s in skin (p=0.02), which was also correlated with collagen content and IL-13 mRNA expression while it remained unchanged in spleen.
Conclusion: We observed a decrease of circulating ILC2s and an increase of ILC2s in SSc skin, correlated with the extent of cutaneous fibrosis. In SSc mice, an increase of skin ILC2 is observed suggesting that these cells could be the trigger of fibrosis in SSc and may constitute a future therapeutic target.
This project is funded by the SFR (Société Française de Rhumatologie) and an ASF (Association des Sclérodermiques de France) grant

P.C6.04.08

Toll-like receptor 3(TLR3) L412F differentially modulates TLR1-9 and non-TLR responses inidiopathic pulmonary fibrosis (IPF) patients: implications for acceleratedisease progression

A. N. McElroy[1], *D. N. O'Dwyer*[2], *A. Tynan*[1], *L. Mawhinney*[1], *P. G. Fallon*[1], *A. G. Bowie*[3], *C. M. Hogaboam*[4], *N. Hirani*[5], *M. E. Armstrong*[1], *S. C. Donnelly*[6];
[1]*School of Medicine, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland,* [2]*School of Medicine and Medical Science, College of Life Sciences, UCD Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland,* [3]*School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland,* [4]*Department of Pathology, University of Michigan Medical School, Michigan, United States,* [5]*Queen's Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom,* [6]*Department of Clinical Medicine, Trinity Centre for Health Sciences, Tallaght Hospital, Dublin, Ireland.*

Introduction: In this study, we investigated the role of defective toll-like receptor 3 (TLR3) and *TLR3* Leu412Phe (*TLR3* L412F; rs3775291) in the pathogenesis of the fatal interstitial lung disease, idiopathic pulmonary fibrosis (IPF). Previously, we established that *TLR3* L412F
was associated with a significantly greater risk of mortality and an accelerated rate of decline in lung function in IPF patients (*AJRCCM* 2013; 180: 1442). Here, we investigated the effect of *TLR3* L412F on responses of primary human lung fibroblasts from IPF patients to TLR1-9 and a number of non-TLR agonists.
Materials and Methods: Patients with IPF were genotyped for *TLR3* L412F and its effect was assessed in primary human lung fibroblasts treated with a panel of TLR1-9 agonists and non-TLR agonists. Cytokine, chemokine and type I interferon levels in IPF fibroblasts weredetermined by ELISA and qPCR, respectively.
Results: We demonstrated that *TLR3* 412F reduced NF-κB-induced IL-8 production following activation of: TLR3, TLR4, TLR5, TLR1/2, TLR2/6 and TLR9 in fibroblasts from 412F-heterozygote IPF patients compared with wild-type patients. *TLR3* L412F also attenuated TLR3- and TLR4-induced activation of IRF3-dependent IFN-β and RANTES production in 412F-heterozygote IPF patients. Interestingly, 412F-heterozygote IPF fibroblasts also had attenuated responses to the non-TLR ligands, Poly(dA:dT) and PMA.
Conclusions: This study demonstrates that the effects of *TLR3* L412F are not limited to TLR3 and that *TLR3* L412F can attenuate additional TLR and non-TLR signalling pathways. These findings may have implications for IPF patients during bacterial or viral infection and hence, accelerated disease progression in 412F-heterozygote patients.

P.C6.04.09

Inhibition of peptidyl-arginine deiminases impairs NLRP3 inflammasome assembly and the release of pro-inflammatory IL-1β in macrophages

n. mishra[1], *F. Ahmed*[2], *L. Ghebremariam*[1], *M. M. Lerch*[3], *R. E. Schmidt*[4], *L. Bossaller*[1];
[1]*Section of Rheumatology, Department of Medicine A, Greifswald, Germany,* [2]*Department of Dermatology, University of Heidelberg, Heidelberg, Germany,* [3]*Department of Medicine A, University Medicine Greifswald, Greifswald, Germany,* [4]*Department of Clinical Immunology and Rheumatology, Hannover Medical School, Hannover, Germany.*

Inflammasomes are cytosolic pattern recognition receptors of the innate immune system. They assemble into multi-protein complexes secondary to the recognition of pathogenic stimuli as well as host derived molecules. The macromolecular aggregation of the receptor protein Nod like receptor (NLRs) with the adaptor protein ASC and the effector protein caspase-1 through homotypic domain-domain interactions into 'ASC specks' controls the release of proinflammatory cytokine IL-1β. Of the host derived molecules, high extracellular calcium concentrations or calcium mobilization from endoplasmic reticulum stores triggers NLRP3 inflammasome activation in macrophages. Peptidyl-arginine deiminase (PAD) are class of calcium dependent enzymes that catalyze the post-translational modification of arginine residues into citrulline amino acid. Of the five known PAD isoforms, only PAD2 and PAD4 are expressed in macrophages.
High intracellular calcium concentration is essential for PAD activity that can be reached in dying cells. We examined protein citrullination and role of PAD enzymes in inflammasome activation. We report here that protein citrullination is common following NLRP3 inflammasome activation in murine macrophages. Interestingly, ASC specks are citrullinated as well. Furthermore, PAD enzyme inhibition resulted in a dose-dependent reduction in the release of active caspase-1 and IL-1β in macrophages. Consistently, we observed a reduction in ASC speck formation following PAD inhibition. Genetic deficiency of PAD-4 alone was not sufficient to block IL-1β release. However, siRNA knockdown of PAD-2 within PAD-4$^{-/-}$macrophages blocked IL-1β release. In conclusion, we find that PAD enzymes fulfill a previously unknown role in NLRP3 inflammasome assembly and IL-1β maturation.

P.C6.04.10

Polyunsaturated omega-3 fatty acids have immunosuppressing effects on NK cell phenotype *in vitro*

S. Y. Omarsdottir[1,2], *K. N. Jensen*[1,2], *J. Freysdottir*[1], *I. Hardardottir*[2];
[1]*National University Hospital of Iceland, Reykjavik, Iceland,* [2]*University of Iceland, Reykjavik, Iceland.*

Dietary fish oil, rich in omega-3 polyunsaturated fatty acids (n-3 PUFA), enhanced resolution of antigen-induced inflammation in mice and induced an early increase in the number of NK cells at the inflamed site, indicating that NK cells may play a role in the resolution of inflammation. The objective of this study was to examine the effects of n-3 PUFA on NK cells *in vitro*. NK cells were isolated from buffy coat using MACS negative selection. They were cultured for 18 hours with/without 50 μM of the omega-3 PUFA EPA or DHA, or with the omega-6 PUFA arachidonic acid (AA), and then stimulated with IL-2, IL-12 and IL-15 for 24 hours. Cytokine concentration in the medium was measured by ELISA and expression of surface markers by flow cytometry. NK cells cultured with DHA secreted less TNF-α (14%), CCL3 (17%) and CCL20 (44.5%) than NK cells cultured without fatty acids. NK cells cultured with DHA also had lower mean expression of the surface markers CX_3CR1 (14%), NKG2A (6.3%) and CXCR3 (5%). NK cells cultured with EPA secreted less TNF-α (13.5%) but more IFN-γ (12%) than NK cells cultured without fatty acids. DHA decreased NK cell secretion of neutrophil recruiting cytokines and chemokines (TNF-α, CCL3 and CCL20) and decreased NK cell expression of chemokine receptors (CX_3CR1 and CXCR3) that mediate migration of immune cells to the inflammation site. These results indicate that n-3 PUFA may modulate NK cell activity and thereby affect inflammation and its resolution.

P.C6.04.11

The cytotoxic function of NK cells in immune disorders is regulated by CD69

M. Relaño Orasio[1], *A. Tsilingiri*[1], *C. Torroja*[1], *M. J. Gómez*[1], *A. Angulo*[2], *C. Muñoz*[3], *C. Schwärzler*[4], *M. Toulson*[4], *P. Martín*[1];
[1]*Spanish National Center for Cardiovascular Research, Madrid, Spain,* [2]*Faculty of Medicine, Barcelona University, Barcelona, Spain,* [3]*La Princesa Hospital, Madrid, Spain,* [4]*Ecole Polytechnique Fédéral de Lausanne, Lausanne, Switzerland.*

Introduction: CD69 is a C-type lectin family receptor that is expressed upon activation and exerts anti-inflammatory effects. Previous works have shown that *Cd69-/-* mice are resistant to tumour growth and vaccinia virus infection in part due to NK cells activity. However, the specific contribution of these cells and the mechanism by which CD69 could be regulating their function has not been described. The activation of NK cells depends on the balance between activating/inhibitory signals. Thus, in this work we study the membrane receptor repertoire of *Cd69-/-* NK cells and it's regulation by CD69. Finally we propose anti-CD69mAbs treatment as a potential therapy in GvHD.
Materials and methods: We used the new mass cytometry (CyTOF) technology to perform an unbiased multiparametric phenotyping of *Cd69-/-* NK cells and using different animal models we studied the *in vivo* consequences of this phenotype in contexts such as anti-viral and anti-tumour immunity and GvHD resistance (allogenic cells recognition).
Results: Our multiparametric study revealed that *Cd69-/-* NK cells present an altered inhibitory/activating receptor repertoire with higher levels of non-self and missing self recognition receptors. This makes them more efficient in the killing of allogenic and tumour cells, being Cd69-/- mice resistant to aGvHD. All these effects can be reproduced by an anti-CD69 mAb treatment.
Conclusions: Cd69-/- NK cells are more efficient in the killing of tumour and allogenic cells due to different expression of activating/inhibitory receptors. This study suggests that anti-CD69mAb treatment could be employed in the clinics to reduce GvH effects potentially maintaining GvL effects.

P.C6.04.12

Identification of different subsets of ILC group I in the healthy and inflamed murine CNS

S. Romero Suarez[1], *A. del Rio Serrato*[1], *D. Brunotte-Strecker*[1], *L. Hertwig*[2], *C. Infante Duarte*[1];
[1]*Institute for Medical Immunology, Charité Universitätsmedizin Berlin, Germany,* [2]*Center for Infectious Medicine, Karolinska Institute, Stockholm, Sweden.*

Natural killer (NK) cells are emerging as important regulators of autoimmune inflammation in multiple sclerosis and its animal model the experimental autoimmune encephalomyelitis (EAE). They have been recently reclassified as part of innate lymphoid cells (ILCs) group I. The helper like ILC1 reside in the mucosae while the cytotoxic ILC1 are represented by the circulating conventional (c)NK cells. In addition, tissue resident (tr)NK cells have been described in various organs like liver and uterus and share properties with mucosal ILC1s and cNK cells. We investigated the CD3-NK1.1+ NK cells present in the CNS of the C57/Bl6 mouse during steady state conditions and identified three distinct NK populations based on the expression of the integrins CD49a and CD49b. Upon the analysis of the expression of their transcription factors, cNK cells were defined as CD49a-CD49b+ Tbet+ Eomes+; trNK cells as CD49a+CD49b-Tbet+Eomes-; and the intermediate population (intNK) as CD49a+CD49b+Tbet+Eomes+/-. The three NK cells subsets were further characterized by flow cytometry analysis. Next, we investigated the response of these populations to autoimmune inflammation of the CNS using the EAE mouse model. Our data show a differential activity of the three populations in their proliferative response, IFN-γ and TNF-α production and chemokine receptor, death ligand and activation markers regulation during EAE. In sum, our data show for the first time the presence and response to inflammation of tissue resident NK cells of the CNS.

P.C6.04.13

Circulating extracellular vesicles from patients with granulomatosis with polyangiitis stimulate neutrophils to generate reactive oxygen species and neutrophil extracellular traps

M. Surmiak[1], *A. Gielicz*[1], *D. Stojkov*[2], *R. Szatanek*[3], *K. Wawrzycka-Adamczyk*[1], *S. Youssefi*[2], *H. Simon*[2], *J. Musial*[1], *M. Sanak*[1];
[1]*Department of Internal Medicine, Jagiellonian University Medical College, Krakow, Poland,* [2]*Institute of Pharmacology, University of Bern, Bern, Switzerland,* [3]*Department of Clinical Immunology, Jagiellonian University Medical College, Krakow, Poland.*

Introduction Activation of neutrophils is a one of the key mechanisms observed in pathology of granulomatosis with polyangiitis (GPA). In this study we evaluate if extracellular vesicles circulating in plasma of GPA patients can contribute to this process. **Material and methods** Extracellular vesicles (EV) from plasma of GPA patients in active stage of the disease (n=10) and healthy controls (n=10) were isolated by ultracentrifugation and characterized by flow cytometry (CD9, CD63, CD81 expression) or nanoparticle tracking analysis. Targeted oxylipins lipidomics of EV was performed by LC-MS. Neutrophil extracellular traps formation (NETs) by neutrophils stimulated with EV or oxylipins was analyzed by fluorescent microscopy or Pico Green assay. ROS production and neutrophils EV binding/ uptake were evaluated by flow cytometry. **Results** EV isolated from plasma of GPA patients stimulated neutrophils to produce reactive oxygen species and release of mitochondrial DNA. However this was observed only when neutrophils were primed with GM-CSF. Priming with GM-CSF increased neutrophils EV binding/ uptake as well. Extracellular vesicles isolated from plasma of GPA patients had higher concentration of leukotriene B4 (LTB4) and 5-oxo-eicosatetraenoic acid (5-oxo-ETE) comparing to EV from healthy controls. Moreover, neutrophils stimulated with these oxylipins (LTB4 or 5-oxo-ETE) responded in ROS and NETs production in a concentration dependent manner. **Conclusions** Presented results reveal the potential of extracellular vesicles to activate neutrophils. ROS production and NET formation by stimulated neutrophils is probably linked to EV oxylipins cargo. The study was supported by National Center of Science in Poland, grant number: 2016/21/D/NZ6/02123

P.C6.04.14

The presence and characterization of Mucosal Associated Invariant T-cells in renal tissue

M. L. Terpstra[1,2], *M. J. Sinnige*[2], *E. Remmerswaal*[2], *M. C. Van Aalderen*[2], *J. Kers*[3,4], *S. E. Geerlings*[5], *F. J. Bemelman*[1];
[1]*Renal Transplant Unit, Division of Nephrology, Department of Internal Medicine, Academic Medical Center, Amsterdam, Netherlands,* [2]*Department of Experimental Immunology, Academic Medical Center, Amsterdam, Netherlands,* [3]*Department of Pathology, Academic Medical Center, Amsterdam, Netherlands,* [4]*University of Amsterdam, Van 't Hoff Institute for Molecular Sciences (HIMS), Amsterdam, Netherlands,* [5]*Division of Infectious diseases, Department of Internal Medicine, Academic Medical Center, Amsterdam, Netherlands.*

Introduction Mucosal Associated Invariant T (MAIT) cells are innate-like T-cells involved in the antibacterial and fungal response by recognizing riboflavin metabolites produced by these organisms. MAIT cells are present in human blood and are highly abundant in the mucosa of the liver, lungs and intestines. It is unclear whether MAIT cells are present in renal tissue and how they phenotypically compare to circulatory MAIT cells.
Methods We used a fluorescently-labelled MR1-tetramer in conjunction with 14-color flowcytometry to identify and characterize MAIT cells in healthy tissue collected from kidneys surgically removed because of renal cell carcinoma (adjacent non-tumorous tissue) (n=5), in renal allografts explanted after allograft failure (n=14) and in blood from healthy donors (n=9).
Results The mean percentage of MAIT cells within the lymphogate was 0.11% (control kidneys) vs 1.10% (renal allografts) and 0.93% (blood) ($p>0.05$). Due to MAIT cell counts <25 (predefined cutoff value) characterization of MAIT cells was impossible in the control kidneys.
MAIT cells in renal allografts appeared to have a relative cytotoxic, activated and tissue resident profile compared to the healthy blood samples, with a significantly higher expression of granzyme B, Ki-67 and CD69/CD103 ($p<0.01$) and a lower expression of CD95, CD127, CD161 and KLRG1 ($p<0.01$).
Conclusion MAIT cells can be detected in renal tissue. Though non-significant, MAIT cells percentages seemed to be higher in renal allografts than in healthy renal tissue. MAIT cells in renal allografts consist of a distinct population with a different expression profile than MAIT cells that are present in healthy blood.

P.C6.04.15

Altered phenotype and cytotoxic potential of NK cells in patients with systemic juvenile idiopathic arthritis and macrophage activation syndrome

J. Vandenhaute[1], *M. Imbrechts*[1], *K. Put*[1], *O. Rutgeerts*[2], *L. De Somer*[3], *C. Wouters*[3], *P. Matthys*[1];
[1]*Rega Institute for Medical Research, KULeuven, Leuven, Belgium,* [2]*Laboratory of Experimental Transplantation, KULeuven, Leuven, Belgium,* [3]*Pediatric Rheumatology, University Hospitals Leuven, Leuven, Belgium.*

Introduction. Systemic juvenile idiopathic arthritis (sJIA) is a severe immune-inflammatory childhood disease characterized by arthritis and systemic features. About 10% of sJIA patients develop macrophage activation syndrome (MAS), a life-threatening hyperinflammatory cytokine storm syndrome characterized by excessive activation of T cells and macrophages.
Objective. sJIA and MAS are linked to defects in cytotoxicity of NK cells. In this study we investigated the phenotype and cytotoxic potential of NK cells in sJIA and MAS patients.
Results. Extensive phenotypic analysis of blood cells from active and inactive sJIA and active MAS patients revealed a decreased expression of cytotoxic proteins (i.e. perforin and granzyme K) and alterations in the expression of inhibitory and activating receptors, with decreased expression of CD57 and Killer Immunoglobulin Receptors (KIR) and increased levels of NKp44 by NK cells. In addition, the activation status and expression of ligands for NK cell receptors was analyzed on monocytes of sJIA and MAS patients. To investigate the cytotoxic potential of NK cells against autologous activated monocytes we optimized a protocol, which allows us to compare NK-specific cytotoxicity against K562 tumor target cells and autologous activated monocytes from patients and healthy controls. sJIA patients showed a defective IL-18-induced IFN-γ expression in NK cells, which is absent in MAS patients and partially resolved in inactive sJIA patients.
Conclusion. NK cells of sJIA and MAS patients show an altered phenotype. Subtle defects in their IFN-γ production and autologous killing of activated immune cells may underlie the immune-inflammatory dysregulation in sJIA.
Research funded by FWO.

P.C6.04.17

IMMUNOPATHOGENESIS OF GESTACIONAL DISORDERS AND PATHOLOGY OF FETUS /NEWBORN BABY THYMUS

R. Sepiashvili[1], *E. Levkova*[2], *L. Dilanyan*[1];
[1]*Peopels Friendship University of Russia,RUDN University, Moscow, Russian Federation,* [2]*RUDN University, Moscow, Russian Federation.*

In the conducted studies, mechanisms of impaired immunological tolerance in the fetus / newborn baby are shown. Sharp changes in cellular composition were characteristic the threat of interruption of pregnancy and OPG - gestosis 3 rd group. IRI in the both groups was 1.03 ± 0.11 and 1.32 ± 0.06, respectively, compared with the 1st group - 0.92 ± 0.04. In the thymus in newborn children of the 1 st group, the cells with the phenotype CD25 +, CD16 + were not detected. In the 2nd and 3rd groups, the level of designated cell markers was higher ($p<0.05$). Such cytokines as IL-1, IL-2, TNF-alpha, IL-8, interferons alpha and gamma were sharply increased ($p<0.05$) in the 2nd and 3rd groups compared to the 1st group. The abolition of the suppressor dominant was indicated by the values of IL-10 and IL-4, which in the comparison groups were 0.13 ± 0.05, 47.43 ± 2.9 pg / ml and 0.09 ± 0.009, 25.65 ± 2, 41 pg / ml, respectively, compared with the 1 st group - 0.82 ± 0.28 and 49.14 ± 5.49 pg / ml. The confirmation of the damage of the thymus was the reaction of the accidental transformation of the thymus, which reached its irreversible degree IV in the 2 nd group, in the 3 rd to the 3 rd degree, in the 1 st group - the III and IV stages of the accidental transformation were fixed.This publication was prepared with the support of the "RUDN University Program 5-100".

P.C6.04.18
ETIOPATHOGENETIC AND IMMUNOLOGICAL CHARACTERISTICS OF INFERTILITY

***R. Sepiashvili**, E. Levkova, T. Slavyanskaya;*
Peopels Friendship University of Russia,RUDN University, Moscow, Russian Federation.

It was shown that the predominant majority of married couples were identified by the HLA-DRB1 gene, which was more often associated with herpesvirus infections. Despite the fact that the level of PCR-positive results in the group of married couples with primary infertility was higher than in married couples with secondary sterility (100 and 92%, respectively), the maximum values of titres of specific antibodies to herpesviruses, including the detection rate of markers Exacerbations of infection (IgM antibodies) in the group of married couples with secondary sterility were higher than in the group of married couples with primary infertility, and significantly higher than in married couples with normal fertility. Therefore, it is this histocompatibility antigen - HLA-DRB1 - that is associated with a significant frequency of sterility and infectious contamination with herpesviruses (HSV 1, 2 types, CMV, VEB) in married couples with secondary infertility. The marker HLA-DRB1, most often found in married couples with infertility, including idiopathic, is associated in this contingent not only with significant contamination with herpesviruses, but also with the presence of a chronic infectious process in the reactivation stage. Conducted clinical and immunological studies clearly demonstrate the need for a comprehensive examination of women with functional disorders in the immunogenesis system with mandatory assessment of the level of infectious (virus) contamination and allows us to propose a comprehensive examination algorithm for functional disorders in the immunogenesis system, including the study of the level of the viral load as a necessary component.This publication was prepared with the support of the “RUDN University Program 5-100”.

P.C6.04.19
Evidence of the NLRP3 Inflammasome-Complement Axis in Osteoarthritis

*J. Bramhall, C. Bessant, J. Neisen, **I. Osuch**, A. Dawson, K. Triantafilou, E. Nichols;*
GlaxoSmithKline, Stevenage, United Kingdom.

Osteoarthritis (OA) has long been considered a “wear and tear” disease, but recent findings suggest that chronic low-grade inflammation plays a role in its pathogenesis. The synovial tissue has also taken centre-stage as target and producer of inflammatory stimuli. Evidence from patient and rodent studies strongly implicates complement in OA pathology. However, the downstream mechanisms have not been elucidated. An improved mechanistic understanding will enable design of therapeutic strategies. This study aimed to explore how complement may mediate synovial inflammation.
Analysis of patient tissues (synovial fluid, synovium) confirmed complement activation in OA. Membrane attack complex (MAC) deposition and increased IL-18 suggest involvement of the NLRP3 inflammasome. This led to the hypothesis that chronic inflammation in OA may depend on a synergistic complement-inflammasome interaction.
To validate the patient data, we examined MAC-mediated inflammasome activation in cell lines and primary synovium. Sub-lytic MAC challenge of the synovial membrane biopsies and synovial cell line resulted in an IL-1β response, suggesting a complement-inflammasome interaction. This response was dependent on both C5, NLRP3 and PI3K. Our data suggests that C5a provides a priming signal for inflammasome activation. MAC was found to provide signal 2, but is also able to trigger inflammasome activation independently of C5a.
In conclusion, terminal pathway effectors can trigger both signals of inflammasome activation, providing a mechanism in which the terminal pathway of complement may perpetuate inflammation in OA.
(Human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.)

P.C6.04.20
StemBell therapy stabilizes atherosclerotic plaques after myocardial infarction

*L. Woudstra[1,2], **A. van Broekhoven**[1,2,3], E. Meinster[1], M. Koopman[1], M. C. Morrison[4], A. C. van Rossum[5,2], M. N. Helder[6], L. J. Juffermans[5,2], H. W. Niessen[1,2,3], P. A. Krijnen[1,2];*
[1]Department of Pathology, VU University Medical Centre, Amsterdam, Netherlands, [2]Amsterdam Cardiovascular Sciences, Amsterdam, Netherlands, [3]Department of Cardiothoracic Surgery, VU University Medical Center, Amsterdam, Netherlands, [4]Department of Metabolic Health Research, The Netherlands Organization for Applied Scientific Research (TNO), Leiden, Netherlands, [5]Department of Cardiology, VU University Medical Centre, Amsterdam, Netherlands, [6]Department of Oral & Maxillofacial Surgery, VU University Medical Centre, Amsterdam, Netherlands.

Background: Myocardial infarction (MI) accelerates atherosclerosis through increased plaque inflammation and destabilization resulting in an increased risk of recurrent MI. Mesenchymal stem cells (MSC) are a promising therapeutic option for atherosclerosis. Here we investigated the effect of StemBell therapy (MSC-microbubble complexes combined with ultrasound) on atherosclerotic plaques after MI.
Methods: MI was induced in atherosclerotic ApoE$^{-/-}$ mice. Six days post-MI, intravenous StemBells or vehicle were given, followed by 1 minute of transthoracic ultrasound. The effects of StemBell treatment on plaque size and stability and the infarcted heart were determined 28 days post-MI. Moreover, monocyte subtypes and lipids in the blood were studied.
Results: StemBell treatment significantly increased cap thickness, decreased intra-plaque macrophage density and increased the percentage of intra-plaque anti-inflammatory macrophages. Plaque size and serum cholesterol and triglycerides were not affected. Furthermore, StemBell treatment significantly increased the percentage of anti-inflammatory macrophages within the infarcted myocardium, but did not affect cardiac function nor infarct size. Finally, the percentage of anti-inflammatory monocytes in the circulation was increased after StemBell therapy.
Conclusion: Systemic StemBell therapy decreased plaque inflammation and destabilization, predominantly associated with local and systemic effects on macrophages/monocytes. Hence, StemBell therapy may be a therapeutic option to prevent atherosclerosis acceleration after MI.
This study was funded by ZonNW (Translational Adult Stem Cell Research program, grant number 116005003) and by a grant from the ICaR-VU institute, Amsterdam, The Netherlands.

P.C6.05 Innate control of inflammation and tissue repair - Part 5

P.C6.05.02
Arginine metabolism during Behçet disease : correlation with the clinical expression

***H. Belguendouz**[1], M. L. Ahmedi[1], A. Chekaoui[1], K. Lahmar[1], D. Hakem[2], Z. Djeraba[1], F. Otmani[3], C. Touil-Boukoffa[1];*
[1]Cytokines and NO Synthases team, LBCM, USTHB, Algiers, Algeria, [2]Internal medicine department, CHU Bab ElOued, Algiers, Algeria, [3]Internal medicine department, CHU Mustapha Bacha, Algiers, Algeria.

Behçet disease (BD) is a chronic systemic inflammatory disorder with uncertain etiology. In previous studies, we showed an increase in nitric oxide (NO) production during disease active stages. NO is the product of NO synthases. It is synthetized from L-Arginine. However, this amino-acid is also the substrate of other enzymes. In this study, we investigated the activity of the other enzymes implicated in arginine metabolism.
65 patients fulfilling BD diagnosis criteria were included in the study (35 active and 30 inactive) and 25 healthy controls (HC). The activity of NOS, ARG, ADC, AGAT and GAMT were assessed through their products measurement. IL1β and IL-27 were measured by ELISA. Mann Witney U test was used for statistical comparison. Spearman test was used for correlation study.
Our results showed a significant increase in all enzymes activity during BD in comparison to HC ($p<0.05$). In addition, we observed a significant increase of NOS and ADC activities and IL-1β during active BD in comparison to inactive BD ($p<0.01$) or HC ($p<0.001$). Also, NOS and ADC showed increased activities during inactive BD in comparison to HC ($p<0.05$). Correlation analyses showed no significant correlations between the different enzymes however a significant positive correlation was observed between NOS and IL-1β ($r=0.62$, $p<0.05$) while a negative correlation was observed between IL-27 and arginase ($r= -0.45$, $p=0.021$).
In conclusion, our results showed an increased activity in arginine metabolism during BD especially NOS and ADC in relation with disease activity. Moreover, this activity seems to be related to cytokines production.

P.C6.05.03
Gastro protective and anti inflammatory activities of *Pistacia lentiscus L.* extracts against gastric ulcer in rats

***I. BOUTEMINE**[1], M. Amri[1], Z. Amir[2], C. Fitting[3], J. Cavaillon[3], C. Touil-Boukoffa[1];*
[1]University of Sciences and Technology Houari Boumediene, algiers, Algeria, [2]Pathology department, Mustapha Pacha Hospital, Algiers, Algeria., algiers, Algeria, [3]Cytokines & Inflammation, Pasteur Institute, Paris, France.

Aim of the study: In the present study, we investigated the anti-ulcerogenic and anti-inflammatory activities of fatty oil (PLFO) and aqueous extract (PLAE) of *Pistacia lentiscus* on ethanol-induced gastric ulcers in Wistar rats. **Material and methods:** PLFO and PLAE were orally administered to rats before gastric ulcer induction by ethanol. The lesions of the gastric mucosa were evaluated by macroscopic and histopathological examination. In addition, the amount of nitric oxide (NO) and pro-inflammatory cytokines (tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6)) were assessed in the plasma and the supernatant of explants cultures of gastric mucosa. Finally, the mucus production and iNOS (inducible NO synthase) expression were determined by histochemical and immunohistochemical analysis respectively. **Result:** Our results indicated that the pretreatment with PLFO and PLAE significantly reduced the areas of gastric’ ulceration and hemorrhage. Interestingly, pretreatment with these extracts highly reduced the plasmatic concentration of NO.

In addition, a significant decrease of NO, IL-6 and TNF-α levels was observed in explants culture. Moreover, iNOS expression was also reduced in the gastric mucosa. In contrast, mucus production by goblet cells was enhanced. Interestingly, the histological analysis of the gastric mucosa has indicated that PLFO and PLAE pretreated groups displayed normal histology. **Conclusion:** Our results demonstrate that PL extracts display significant prophylactic effects against gastric ulcer. Importantly, the mechanism underlying PLFO and PLAE activities might implicate the inhibition of inflammatory responses during gastric ulcer.

P.C6.05.04

Engagement of Fas differentially regulates the production of LPS-induced pro-inflammatory cytokines and type I Interferons

*C. Lyons[1], K. Brennan[2], S. Doyle[3], A. Houston[1], **E. Brint**[1];*
[1]University College Cork, Cork, Ireland, [2]Trinity College Dublin, dublin, Ireland, [3]Trinity College Dublin, Dublin, Ireland.

Best known for its role in apoptosis, recent reports suggest that Fas (CD95) signalling is also involved in other cellular responses including inflammation. Whilst Fas and its adaptor protein FADD have been previously shown to negatively regulate LPS-induced pro-inflammatory responses, their role in LPS-induced type-1 Interferon production is unknown. Here we demonstrate that Fas engagement on THP-1 macrophages using an agonistic Fas antibody CH11, augments LPS-induced NF-κB responses, causing an increase in the production of TNFα, IL-10, IL-8, IL-6 and IL-12. Conversely, co-stimulation with both LPS and CH11 causes a significant reduction in the level of IFNβ production. This differential effect involves the Fas adaptor FADD as, whilst LPS-induced IL-6 production was increased in $FADD^{-/-}$ murine embryonic fibroblasts, LPS-induced IFNβ production was significantly reduced in these cells. Overexpression of a dominant negative form of FADD, the FADD-Death Domain (FADD-DD), in the RAW264.7 macrophage cell line, inhibits LPS-induced IFNβ-luciferase but not LPS-induced NF-κB luciferase. In contract, overexpression of full-length FADD inhibited LPS-induced NF-κB-luciferase activation but was seen to augment LPS-induced IFNβ-luciferase. Moreover, the FADD-DD inhibits TLR-4-, TRIF- and TRAM-induced IFNβ -luciferase production, indicating that FADD may be interacting with the TLR-4 pathway at the level of these TLR4 adaptor proteins. In conclusion, these data identify FADD as a novel component of the MyD88-independent pathway leading from TLR4 to Type-1 Interferon production and moreover demonstrate that both Fas and its adaptor FADD can differentially regulate the production of LPS-induced pro-inflammatory cytokines and type I Interferons

P.C6.05.05

miRNA-147 targets the electron transport chain component NDUFA4 in response to inflammatory stimuli

***S. A. Clayton**[1,2], S. W. Jones[1], M. Kurowska-Stolarska[3,2], A. R. Clark[1,2];*
[1]University of Birmingham, Birmingham, United Kingdom, [2]Arthritis Research UK Rheumatoid Arthritis Pathogenesis Centre of Excellence (RACE), Glasgow, Birmingham, Newcastle, United Kingdom, [3]University of Glasgow, Glasgow, United Kingdom.

Introduction: MicroRNAs (miRNAs) are an important class of post-transcriptional regulator, with many being described as instrumental in immune cell inflammatory responses. MiRNAs can alter cellular metabolism by targeting essential components of specific metabolic pathways. Macrophage phenotype and function is now known to be intimately linked to cellular metabolic status, and consequently miRNAs have the potential to achieve immunomodulatory effects by targeting macrophage metabolic pathways.
Methods: MiRNA and mRNA expression was measured by RT-qPCR in primary human monocyte derived and mouse bone marrow derived macrophages in response to pro-/anti-inflammatory stimuli. Several miRNA target prediction tools were used (TargetScan, miRanda, miRDB, miRTarBase). NDUFA4 protein expression was detected by Western blotting. Cells were transfected with miRNA mimics (Dharmacon) to validate targets.
Results: Expression of microRNA-147 in macrophages was induced by pro-inflammatory stimuli such as LPS. LPS-induced miR-147 expression was inhibited by the synthetic glucocorticoid dexamethasone in mouse macrophages by up to 93%, and the human homologue miR-147b by up to 81%.
Bioinformatic analysis found that miR-147(b) was predicted to target the 3'UTR of electron transport chain component NDUFA4. Expression of human NDUFA4 mRNA and protein were both significantly downregulated by LPS treatment of macrophages, and NDUFA4 protein expression was rescued by co-treatment with dexamethasone. This is consistent with the regulation of miR-147b by these stimuli. Ectopic expression of miR-147b confirmed that this miRNA functionally targets NDUFA4.
Conclusions: MiR-147 has the potential to regulate macrophage metabolism in response to pro-inflammatory signals by downregulating a key electron transport chain component.
Arthritis Research UK grant 20298

P.C6.05.06

ERAP1 depletion mimics the effects of the Behçet's Disease associated enzyme variant on the HLA-B*51 peptidome

***P. Guasp**[1], M. Compagnone[2], E. Barnea[3], J. J. Kuiper[4], D. Fruci[2], A. Admon[3], J. López de Castro[1];*
[1]Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain, [2]Immuno-Oncology Laboratory, Pediatric Haematology/Oncology Department, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy, [3]Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel, [4]Department of Ophthalmology, University Medical Center Utrecht, Utrecht, Netherlands.

The Endoplasmic Reticulum Aminopeptidase ERAP1 trims peptides to the correct length for presentation by HLA class I molecules. HLA-B*51 shows a high preference for peptides that carry Proline or Alanine at position 2. The Pro2 subpeptidome shows higher affinity for HLA-B*51 than the Ala2 subpeptidome.
ERAP1 was knocked out in the transfectant cell line 721.221-HLA-B*51 using the CRISPR Cas9 technology. The B*51 bound peptidome presented by the WT and ERAP1 KO cells was comparatively analyzed by quantitative mass spectrometry, and the surface expression of HLA-B*51 was measured by flow cytometry. The theoretical affinity of the identified peptides for HLA-B*51 was calculated using predictive algorithms.
The absence of ERAP1 drastically altered the ratio between the Ala2 and Pro2 subpeptidomes. This effect dramatically reduced the affinity of the peptidome presented by the ERAP1 KO cells due to the overrepresentation of the low affinity subpeptidome of Ala2 ligands. ERAP1 KO did alter neither the surface expression of HLA-B*51 nor the levels of Free Heavy Chain in this transfectant cell line.
Overall, we demonstrate that ERAP1 is required to maintain the balance between Ala2 and Pro2 ligands in HLA-B*51 cells, and its absence leads to a peptidome of much lower affinity. The effects of ERAP1 depletion on the HLA-B*51 peptidome are very similar to those observed in the presence of the low activity and Behçet's associated ERAP1 Hap10 variant. Being the interaction between HLA-B*51 and KIR3DL1 sensitive to the HLA-B*51 peptidome, it is very likely that ERAP1 depletion may affect the NK cell reactivity.

P.C6.05.07

IgA versus IgG: the potential and danger of IgA as potent immune cell activator

***M. H. Heineke**;*
VU medical centre, Amsterdam, Netherlands.

Antibody-opsonized pathogens can activate immune cells via Fc receptors. Both IgA Fc receptor (FcαRI) and IgG Fc receptor IIA (FcγRIIA) are thought to initiate similar signaling pathways and responses, but we previously showed that only IgA triggering of neutrophils led to leukotriene B4 release with concomitant neutrophil migration. In this study we investigated cellular activation through IgA or IgG in more detail using different methods, including live cell imaging, (phospho)proteomics and metabolomics. No differences were observed in uptake of IgG- or IgA-coated beads and subsequent release of reactive oxygen species and neutrophil extracellular traps. However, crosslinking of FcαRI led to a slower but stronger and more sustained signaling profile, exemplified by increased intracellular calcium and phosphotyrosine levels. Only IgA stimulation induced downstream events, like release of cytokines, chemokines and pro-inflammatory lipids. Importantly, enhanced activation through FcαRI is not neutrophil specific, as stimulation of monocytes with IgA also led to increased activation. These results support 1) that signaling routes of FcγRIIA differ from those that are initiated by FcαRI, resulting in distinct functional profiles, and 2) IgA is a more potent activator of immune cells than previously anticipated. This may have significant implications in autoimmunity, as IgA auto-antibodies are found in a multitude of autoimmune diseases. Moreover, IgA may represent a potent novel immune activator during cancer immunotherapy.

P.C6.05.08

Lunasin regulates obesity-related inflammation in C57BL/6 mice fed with high fat diet

***C. C. Hsieh**[1,2], M. J. Chou[2], S. H. Peng[2];*
[1]School of Life Science, Programs of Nutrition Science, National Taiwan Normal University, Taipei, Taiwan, [2]Department of Human Development and Family Studies (Nutritional Science & Education), National Taiwan Normal University, Taipei, Taiwan.

Accumulating evidence has shown that extra adiposity influences the progression of various chronic diseases. The process of adiposity induces the infiltration of macrophages, accompanied by multiple inflammatory mediators. Lunasin, a natural seed peptide, exhibits several biological activities, such as anti-carcinogenesis, anti-inflammatory, and antioxidant activities. The aim of this study is to investigate whether lunasin regulates obesity-related inflammation in mice fed with high fat diet. C57BL/6J mice were fed a low fat (LF), or high fat (HF) diet, and HF intraperitoneal injected by lunasin 4 mg/kg bw (HF-LL) and 20 mg/kg bw (HF-HL) for 7 weeks. Our results showed that the HF mice have significant higher body weight and organs weight including spleen, liver, and adipose tissue than the LF mice. The oxidized lipid malondialdehye value was significantly higher in the HF group. The mice received lunasin significantly decreased malondialdehye values in the adipose and liver. Interleukin (IL)-1β production by LPS-stimulated peritoneal cells was increased in HF mice, and were decreased in mice received lunasin treatment.

Histological analysis of the epididymal adipose tissue of HF groups with lunasin treatment showed less F4/80+ macrophage infiltration, especially lower CD11c+M1 phenotype, compared to HF and LF groups. More related mediators should be analyzed in the future. In summary, lunasin regulates obesity related mediators in C57BL/6 mice fed with high fat diet, suggesting the intake of lunasin from diet or a supplement, for auxiliary prevention or therapy in obesity-related applications. The authors acknowledge the funding from Ministry of Science and Technology, Taiwan (MOST 106-2311-B-003-005-MY3).

P.C6.05.09

Frequency and activation status of myeloid cells in the Guillain-Barre syndrome

W. van Rijs, W. Fokkink, A. Tio-Gillen, M. Brem, B. Jacobs, ***R. Huizinga****;*
Erasmus MC, University Medical Center, Rotterdam, Netherlands.

The Guillain-Barré syndrome (GBS) is an acute immune-mediated neuropathy, which may develop after relatively common infections, including *Campylobacter jejuni* and cytomegalovirus. Macrophages are important in the pathogenesis of GBS by phagocytosing myelin and axons in the nerve. However, little is known about their precursors in the peripheral blood. Here we assessed the composition and phenotype of monocytes and dendritic cell (DC) subsets in peripheral blood of GBS patients. Peripheral blood mononuclear cells (PBMC) were isolated from GBS patients (n=20), before and after immunomodulatory treatment, and age and gender-matched, healthy controls (n=20). The frequency and phenotype of six myeloid cell subsets was determined by 13-color flow cytometry. The frequency of total monocytes, determined as percentage of CD45+ cells, was significantly increased in GBS patients compared to controls. The monocyte population was skewed towards more intermediate (CD14+CD16+; $p<0.05$) and less non-classical (CD14-CD16+; $p<0.01$) monocytes. Increased expression of CD40, TLR2 and Siglec-7 on non-classical monocytes suggested an enhanced differentiation towards non-classical monocytes. Immunomodulatory treatment strongly reduced the frequency of non-classical monocytes and all DC populations in CD45+ PBMC, as well as expression of CD40, HLA-DR and TLR4 on classical monocytes. In conclusion, our data identify significant changes in the monocyte compartment in GBS. Further analysis should reveal whether these changes are related to preceding infections, disease severity and treatment response.
Supported by the Benson Clinical Research Fellowship (GBS/CIDP Foundation International).

P.C6.05.11

Dietary fish oil enhances the proportion of mature NK cells in murine antigen-induced peritonitis

K. N. Jensen*[1,2], J. Freysdottir[1], I. Hardardottir[2];*
[1]Landspitali - the National University Hospital of Iceland, Reykjavik, Iceland, [2]University of Iceland, Reykjavik, Iceland.

Unresolved inflammatory responses may lead to chronic inflammation, which is linked to the pathogenesis of a number of degenerative diseases in Western countries. We have previously shown that dietary omega-3 PUFA induced an early peak in the number of NK cells in antigen-induced peritonitis in mice and also enhanced the resolution of the inflammation. Furthermore, depletion of NK cells prevented the timely resolution of inflammation in the same model. The aim of this study was to determine the effects of dietary omega-3 PUFA on the NK cells at the peak of the inflammation. Mice were fed either a control or a fish oil enriched diet, immunized with methylated BSA (mBSA) and mBSA injected into the peritoneum. Peritoneal cells were harvested at the peak of inflammation, sorted and assessed by flow cytometry. As was shown in the previous study, mice fed the fish oil enriched diet had a lower number of neutrophils and a higher number of total NK cells at the peak of inflammation. More specifically, mice fed the fish oil diet had a higher number of mature NK cells but a lower number of immature NK cells in their peritoneum than mice fed the control diet. These results suggest that dietary fish oil may enhance maturation of NK cells and that these mature NK cells may play an important role in the resolution of inflammation either by limiting the infiltration of neutrophils or inducing neutrophil apoptosis in a more effective manner.

P.C6.05.12

DNase I treatment ameliorates mouse experimental colitis

E. Y. H. Lin*[1], Y. H. Hsu[1], C. S. Hsueh[2], H. W. Chang[2], H. S. Chiang[1];*
[1]Department of Life Science, National Taiwan University, Taipei, Taiwan, [2]School of Veterinary Medicine, National Taiwan University, Taipei, Taiwan.

Neutrophils are the most abundant white blood cells with potent antimicrobial activities. Neutrophil extracellular trap (NET) is one of the defense mechanisms utilized by neutrophils to control microbial infections. A recent proteome and microscopy analysis of intestinal biopsies of patients with ulcerative colitis indicated an increased neutrophil abundance and aberrant NETs in inflamed colon tissues. However, the impact of NET in colitis has yet been fully illustrated. Here we demonstrated that administrations of DNase I, an endonuclease that dissolves the NET structure, suppressed colitis in a dextran sulfate sodium (DSS) mouse model. DNase I treatments had a tendency to reduce weight loss and clinical symptoms in mice during DSS-induced colitis. DNase I treatments also alleviated DSS-induced colon shrinkage and intestinal architecture disruption. Furthermore, immunofluorescence microscopy revealed a reduced levels of histone H3 citrullination in the colons of DSS-induced colitis mice with DNase I administration. Overall our results suggested that DNase I treatment has the potential capability to alleviate experimental colitis in mice.

P.C6.05.13

Identifying the components of the ubiquitin proteasome system that target the p50 subunit of NF-κB for degradation

J. P. Mitchell*, R. J. Carmody;*
Institute of Infection Immunity and Inflammation, Glasgow, United Kingdom.

Introduction: The NF-κB subunit p50 is a critical regulator of inflammation and is a major transcription factor in most cell types. In macrophages, p50 homodimers act as repressors of transcription to limit the expression of pro-inflammatory cytokines and promote the resolution of inflammation, which is essential for avoiding the damaging effects of prolonged inflammation on the host. The stability of p50 homodimers is an important determinant of this repressor function and is controlled by ubiquitin-triggered proteasomal degradation. Despite its importance, little is known about the molecular mechanisms that target p50 for degradation, or the cellular factors that initiate p50 ubiquitination.
Methods: By using a panel of putative ubiquitin E3 ligases for p50 and a library of p50 mutants containing specific lysine to arginine substitutions, we aim to identify the components of the ubiquitin proteasome system (UPS) that target p50 for ubiquitination and degradation.
Results: Initial data has identified a novel role for SOCS1 as an E3 ligase that ubiquitinates p50. We have also identified a lysine residue of p50 that is targeted for ubiquitination but for which we have not yet identified the specific E3 ligases responsible.
Conclusions: These findings indicate that p50 ubiquitination is orchestrated by a complex network of UPS factors with significant implications for our understanding of this regulatory mechanism in the control of inflammation.

P.C6.05.14

Metabolic alterations in airway macrophages during pulmonary fibrosis

P. P. Ogger*[1], R. J. Hewitt[1], P. L. Molyneaux[1,2], T. M. Maher[1,2], C. M. Lloyd[1], A. J. Byrne[1];*
[1]National Heart and Lung Institute, Imperial College London, London, United Kingdom, [2]NIHR Respiratory Biomedical Research Unit, Royal Brompton Hospital, London, United Kingdom.

Background: Idiopathic pulmonary fibrosis (IPF) is a fatal disease with limited treatment options. Evidence suggests that IPF is in part due to dysregulated wound-healing orchestrated by macrophages. While metabolic reprogramming of macrophages drives inflammation, its contribution to IPF is unknown. We hypothesise that airway macrophage (AM) metabolic reprogramming underlies IPF and that manipulation of AM metabolism represents a potential novel therapeutic strategy for IPF. **Methods:** First we assessed AM number and phenotype in patient bronchoalveolar lavage (BAL) by flow cytometry. Subsequently AMs were enriched and metabolic functionality was analysed by extracellular flux analysis and PCR array of key metabolic enzymes. To investigate the metabolic phenotype of tissue resident and monocyte recruited airway macrophages in pulmonary fibrosis, we utilised the bleomycin mouse model and measured expression of metabolic genes and mitochondrial function in sorted macrophage populations. **Results:** IPF AMs show increased expression of *ENO2* and *IDH2*, while *SDHC* gene expression is decreased and oxygen consumption rate is similar compared to healthy controls. Upon bleomycin treatment, Siglec-F^{int} monocyte recruited airway macrophages gradually replaced Siglec-F^{hi} tissue resident airway macrophages and show higher gene expression of *HK3* and *IDH2*. **Conclusions:** Together these data indicate that during pulmonary fibrosis, AMs undergo metabolic alterations. The differential gene expression of *IDH2* and *SDHC* is particularly interesting considering these are major oncogenes. Future work aims to further characterise the metabolic phenotypes of resident and recruited AMs in the lung and to investigate how alterations in AM metabolic intermediates in IPF may contribute to the disease.

P.C6.05.15

Bosentan therapy modulates miR-21-TGF-β1-bFGF-Let-7d axis and attenuates the development of pulmonary artery hypertension after bleomycin injury

A. Pandey;
Vallabhbhai Patel Chest Institute, Delhi, India.

Introduction: Pulmonary artery hypertension(PAH) develops in 30-40% cases of pulmonary fibrosis. Pathogenesis of PAH is associated with differential expression of miRNAs. MiR-21 inhibits endothelin receptor(ET-B), drives VSMC differentiation and fibrosis. Let-7d binds to endothelin-1and is proangiogenic. The effects of Bosentan, on miRNAs and bFGF, TGF-β1 signaling cascades remain to be elaborated. **Method**: Wistar rats were euthanized on day 7, 14, 28 after bleomycin/saline instillation: GroupI (control, n=18), GroupII (bleomycin, 7 IU/kg, n=18), GroupIII (Bleomycin+Bosentan, 100mg/kg/d, n=18). Let-7d, miR-21, bFGF, TGF-β1 mRNA and protein levels were assessed. **Result:** Bleomycin instillation caused upregulation of miR 21 and downregulation of Let-7d from day 7. An associated upregulation of TGF-β1 and bFGF mRNA and protein levels was seen. bFGF increased in AECs, peribronchiolar fibroblasts and reduced in endothelial cells from day 7. Progressive perivascular inflammation, vasoconstriction and vascular smooth muscle cell hypertrophy(VSMCH) was seen. After bosentan therapy, miR-21 downregulation and Let-7d upregulation was seen on day 28. This correlated with a reduction in TGF-β1 and bFGF mRNA and protein levels, reduction in perivascular inflammation and remodelling while vasoconstriction persisted till 28 day, after bosentan therapy. **Conclusion:** The miR-21-TGF-β1-bFGF-Let-7d axis plays a key regulatory role in the pathogenesis of PAH. TGF-β1 has an autoregulatory feedback loop with miR-21 and bFGF. Loss of endothelial bFGF downregulates Let-7d, increases TGF-β1 and promotes endothelial-mesenchymal transition, leading to VSMCH and vascular remodelling. Bosentan therapy, downregulates miR-21, reduces TGF-β1 signalling, upregulates let 7d and attenuates VSMCH and pulmonary hypertension.

P.C6.05.16

Specialized Pro-Resolving Mediators' receptors expression in peripheral blood derived leukocytes in Rheumatoid Arthritis

***S. Perniola**, L. Dinoia, N. Lacarpia, D. Natuzzi, R. Bizzoca, F. Iannone;*
Rheumatology Unit - Department of Emergence Medicine and Transplantation, Bari, Italy.

Background: Resolution process downregulates the inflammation and promotes tissue repair by the Specialized Pro-Resolving Mediators (SPMs), that act by interacting with specific cellular receptors: CMKLR1, BLT1, FPR2 and GPR32. In rheumatoid arthritis (RA) the reactive inflammation becomes persistent and the innate immune response turns into the adaptive immune activation.
Objectives: nowadays there is no evidence whether SPMs are involved in RA pathogenesis so we evaluated the expression of CMKLR1, FPR2 and BLT1 in RA patients.
Methods: Patients affected with RA were enrolled in this study. At entry, ESR, CRP, DAS28-ESR, CDAI and HAQ were collected. Patients were divided into high-moderate (H-Mo/RA if DAS28-ESR ≥ 3.2) and low-remission (LRem/RA if DAS28 < 3.2) disease activity group. The expression of CMKLR1, FPR2 and BLT1 in peripheral T cells (CD3) and monocytes (CD14) was evaluated by flow-cytometry assay.
Results: Thirty RA patients were studied. SPMs receptors were differently expressed on CD14. BLT1+CD14+ cells were significantly higher in L-Rem/RA than in H-Mo/RA (p: 0.0001). Likewise, FPR2+CD14+ cells were significantly higher in L-Rem/RA than in H-Mo/RA (p: 0.01). We demonstrated an inverse correlation between BLT1 level in monocytes and ESR (p: 0.01), CRP levels (p: 0.008), DAS28-ESR (p: 0.03), CDAI (p: 0,0076) and HAQ (p: 0,0138) and a weak correlation between FPR2 expression and HAQ (p: 0.05).
Conclusions: FPR2 and BLT1 expression seems to be regulated by the activity of RA disease. It might be hypothesized that a defective signalling through these SPMs receptors may contribute to sustain chronic inflammation in active RA.

P.C6.05.17

Surfactant protein D regulates cigarette smoke-induced lung inflammation through inhibition of ceramide synthesis

***B. Pilecki**[1], H. Wulf-Johansson[1], P. Djiadeu[2], C. Støttrup[1], P. T. Jørgensen[1], A. Schlosser[1], S. W. Hansen[1], J. Madsen[3,4,5], H. W. Clark[3,4,5], C. H. Nielsen[6], J. Vestbo[7,8], U. Holmskov[1], G. L. Sørensen[1];*
[1]Department of Cancer and Inflammation Research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark, [2]Program in Translational Medicine, Lung Innate Immunity Research Laboratory, The Hospital for Sick Children Research Institute, Toronto, Canada, [3]Department of Child Health, Sir Henry Wellcome Laboratories, Academic Unit for Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, United Kingdom, [4]Institute for Life Sciences, University of Southampton, Southampton, United Kingdom, [5]National Institute for Health Research, Southampton Respiratory Biomedical Research Unit, Southampton, Centre for Biomedical Research, University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom, [6]Institute for Inflammation Research, Center for Rheumatology and Spine Diseases, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark, [7]Department of Respiratory Medicine, Odense University Hospital, Odense, Denmark, [8]Division of Infection, Immunity and Respiratory Medicine, Manchester Academic Health Science Centre, Manchester University NHS Foundation Trust, Manchester, United Kingdom.

Introduction: Surfactant protein D (SP-D) is a pulmonary collectin with established anti-inflammatory functions. SP-D-deficient mice exhibit pulmonary emphysema together with lipid and alveolar macrophage accumulation. SP-D has been linked to chronic obstructive pulmonary disease (COPD), while exposure to cigarette smoke (CS), main COPD risk factor, upregulates pulmonary SP-D in mice. Ceramides, pro-inflammatory lipid mediators, are known to be involved in CS-induced disease.
Aim: In the current study we hypothesized that SP-D deficiency leads to aggravation of CS-induced lung inflammation.
Methods: C57BL/6N male WT and SP-D-deficient mice were subjected to whole body CS exposure 5 days/week for 12 weeks (chronically) or daily for 3 days (acutely). Recombinant fragment of human SP-D (rfhSP-D) was administered intranasally one hour prior to each CS exposure in the acute regimen.
Results: CS exposure resulted in macrophage-rich airway inflammation that was aggravated in SP-D-deficient mice. In addition, local levels of macrophage inflammatory protein-1 alpha were significantly higher in SP-D-deficient mice than in WT animals after chronic CS exposure and correlated positively with airway total cell count. Furthermore, CS-treated SP-D-deficient mice showed pulmonary accumulation of long-chain ceramides as well as ceramide synthase genes, crucial for ceramide generation. Finally, CS-induced macrophage accumulation and upregulation of ceramide synthase genes could be reversed by rfhSP-D treatment.
Conclusion: Our results indicate that SP-D protects from CS-induced pulmonary inflammation and ceramide generation and that SP-D-based therapy might be a future potential treatment of COPD.

P.C6.05.19

Resident, sessile airway macrophages clear infiltrating neutrophils before monocyte arrival with a minimal neutrophil lymphatic entry

***J. Vuononvirta**[1], D. F. Patel[1], J. Secklehner[2], J. B. Mackey[2], K. De Filippo[1], W. Branchett[1], L. G. Gregory[1], M. B. Headley[3], M. F. Krummel[3], R. J. Snelgrove[1], C. M. Lloyd[1], L. M. Carlin[2];*
[1]Inflammation, Repair & Development, National Heart & Lung Institute, Imperial College London, London, United Kingdom, [2]Cancer Research UK Beatson Institute, Glasgow, United Kingdom, [3]Department of Pathology, University of California, San Francisco, United States.

In the lung, resident airway macrophages (AMs) are often the first immune cells encountered where they provide sentinel function against inhaled pathogens. During infection or inflammation, pro-inflammatory cytokine and chemokine production leads to recruitment of neutrophils into the alveoli to tackle pathogens. During resolution of inflammation, neutrophils are thought to be cleared by airway macrophages to prevent further tissue injury, termed efferocytosis. Since much of our understanding of AM function is based on studies in which AMs were isolated and studied *ex vivo* or depletion studies *in vivo* we lack data on AM behaviour, dynamics and interactions with neutrophils in their natural microenvironment. To address this, and the mechanisms and kinetics of neutrophil clearance during inflammation, we used lung intravital microscopy and imaging of agarose inflated precision cut lung slices in a well characterised LPS mouse model at different time points post instillation (24h, 48h and 72h). Using lung intravital microscopy, we demonstrated that AMs are sessile during steady state and inflammation, but they protrude and retract dramatically post-LPS indicating more active behaviour.
Strikingly, we observed that AMs are able to clear neutrophils efficiently (halving neutrophil numbers in the BAL between 24 and 48 hrs), largely before recruited monocytes arrive by engulfing several neutrophils at a time. Post-LPS, neutrophils are cleared rapidly by AMs with a small percentage entering the lymphatics. Overall, we provide new insights into AM function and behavior during LPS induced lung inflammation *in vivo*.

P.C6.05.20

The absences of Growth Differentiation Factor 15 aggravates adverse cardiac remodeling upon pressure-overload

***M. Wesseling**[1,2,3], J. J. de Haan[1], G. Sanchez-Duffhues[3], L. Bosch[1], M. A. Brans[1], S. J. Lee[4], J. C. Deddens[1], G. Pasterkamp[2,5], J. P. Sluijter[1,5,6], M. J. Goumans[3], S. C. de Jager[1,7];*
[1]Department of Cardiology, Laboratory of Experimental Cardiology, University Medical Center Utrecht, Utrecht, Netherlands, [2]Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, Netherlands, [3]Molecular Cell Biology, Leiden University Medical Center, Leiden, Netherlands, [4]Department Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, United States, [5]Utrecht University, Utrecht, Netherlands, [6]UMC Utrecht Regenerative Medicine Center, Utrecht, Netherlands, [7]Laboratory of Translational Immunology, Department of Immunology, University Medical Center Utrecht, Utrecht, Netherlands.

Background: Growth differentiation factor 15 (GDF15) belongs to the TGF-β family. Under normal conditions, GDF15 is not highly expressed, but sharply increased upon injury. GDF15 influences many processes, such as inflammation, apoptosis and fibrosis. In heart failure (HF) patients, GDF15 plasma levels are increased and high GDF15 levels are associated with increased mortality. Yet, the exact role of GDF15 in adverse cardiac remodeling leading to HF is not known.
Purpose: We therefore studied GDF15 knock-out ($^{-/-}$) mice and wild type (WT) in a pressure-overloaded HF model using transverse aortic constriction (TAC).

Results: After 6 weeks of TAC, GDF15$^{-/-}$ mice have increased end diastolic volume (EDV) and end systolic volume (ESV) (EDV: 93 µl versus 64 µl, p<0.001, ESV: 72 µl versus 38 µl, p<0.001). The accelerated worsening in GDF15$^{-/-}$ mice were already visible 7 days after TAC in both ESV as in global longitudinal strain (-11.8±2.8 % vs. -15.5 ±2.7 %). Immunohistochemistry showed no differences in cardiomyocyte hypertrophy, fibrosis and inflammation. Another mechanism involved in adverse remodeling is endothelial to mesenchymal transition. To assess the role of GDF15 in this process we exposed human primary coronary microvascular endothelial cells to recombinant hGDF15 which resulted in morphological changes representing endothelial to mesenchymal transition. Furthermore protein and reporter assays confirmed this observations.
In conclusion: Lack of GDF15 aggravates adverse remodeling upon pressure-overload. Our data suggests that GDF15 directly induces coronary microvascular dysfunction by endothelial to mesenchymal transition. Further studies will determine to which extent endothelial to mesenchymal transition affects adverse cardiac remodeling.

P.C6.06 Innate control of inflammation and tissue repair - Part 6

P.C6.06.01
The crosstalk between autophagy and inflammation in plasmacytoid Dendritic Cells

C. R. Almeida[1], *C. Silva*[1], *P. Antas*[1], *V. Camosseto*[2], *E. Gatti*[1,2], *P. Pierre*[1,2];
[1]*Institute for Biomedicine – iBiMED, Aveiro, Portugal,* [2]*CIML - Centre d'Immunologie de Marseille-Luminy, Marseille, France.*

Autophagy contributes to cellular homeostasis by eliminating damaged organelles and pathogens, as well as protein aggregates, which might act as inflammasome triggers. Autophagy contributes therefore to proteostasis but also controls inflammation. Dendritic cells (DCs) orchestrate innate and adaptive immunity mostly due to its role as antigen presenting cells and as cytokine producers. Activation of DCs by LPS stimulates the mTORC1 pathway, decreasing the autophagy flux and leading to accumulation of newly synthesized poly-ubiquitinated proteins in dendritic cell aggresome-like induced structures (DALIS). Here we studied the interplay between autophagy, inflammation and protein aggregation on plasmacytoid dendritic cells (pDCs), which are a subset specialized in production of type I interferon (IFN) with an important role in antiviral responses and in the pathogenesis of autoimmune diseases. We found that inhibiting autophagy on the pDC cell line CAL-1 impacts the production of cytokines and protein aggregation. The results suggest a link between autophagy, protein aggregation and inflammation in human pDC. These findings can ultimately be used for future development of novel therapeutic approaches in autoimmune diseases or against infection. This work was supported by Fundação para a Ciência e a Tecnologia and Portugal2020 (FEDER) - reference PTDC/IMI-IMU/3615/2014 - and through POCH (SFRH/BPD/109322/2015).

P.C6.06.02
Efficient engagement of inhibitory receptor ILT4, LILRB2 with complement split products

C. Battin[1], *A. De Sousa Linhares*[1], *J. Hofer*[2], *G. Zlabinger*[1], *J. Leitner*[1], *W. Paster*[3], *P. Steinberger*[1];
[1]*Center for Pathophysiology, Infectiology and Immunology, Vienna, Austria,* [2]*Department of Medicine II, Division of Nephrology and Dialysis, Vienna, Austria,* [3]*CCRI - Children´s Cancer Research Institute, Vienna, Austria.*

The complement system is an evolutionary ancient defense mechanism of innate immunity but plays also an important role in maintaining immune homeostasis during the clearance of immune complexes and apoptotic material. Surfaces of invading pathogens or altered self, trigger complement activation and deposition via a cascade of enzymatic reactions resulting in the release of complement split products CSPs. Deficiencies in C4 are linked to diseases like systemic lupus erythematosus and deposition of the CSP C4d on renal transplants is a strong predictor of graft rejection. Recent data demonstrate a novel interaction between C4d and the inhibitory cell surface receptor immunoglobulin-like transcript 4 (ILT4, also known as LILRB2) present on monocytes and dendritic cells. The major ligands for ILT4 are MHC class I molecules, which inhibit proinflammatory immune responses via the immunoreceptor tyrosine-based inhibitory motifs ITIMs. The exact functional role of C4d and its involvement in the ILT4 signaling pathway is still unknown. The current study therefore investigates the interaction of C4d molecule and other CSP with ILT4. Multimeric C4d molecules were generated to achieve improved receptor engagement. In addition we have developed a novel reporter system to investigate the consequences of ILT4 engagement by engineered C4d molecules without interference by MHC-class I – ILT4 interactions. We anticipate that our data will provide rationales to exploit the immunosuppressive function of C4d in novel therapeutic approaches.

P.C6.06.03
Sepsis induces long-term changes in the transcriptome and epigenome of naïve bone marrow monocytes

K. Bomans*, J. Schenz, D. Schaack, M. A. Weigand, F. Uhle;*
Heidelberg University Hospital, Department of Anesthesiology, Heidelberg, Germany.

"Sepsis-induced immunosuppression" is a typical hallmark of post-septic patients, characterized by a hypo-responsiveness of the host's immune system. This condition renders the host vulnerable to a persisting or the occurrence of secondary, often opportunistic infections, along with increased mortality.
The mechanisms underlying the immunosuppressive phenotype are yet unknown, but the involvement of epigenetic alterations of immune cells seem obvious. Our project aims to unravel these underlying molecular mechanisms.
Epigenetic (ChIP-seq for H3K4me3) and transcriptomic (RNA-seq) analysis of post-sepsis naïve bone marrow monocytes (three months after insult) were analyzed using a cecal ligation and puncture (CLP) mouse model of polymicrobial abdominal sepsis. Also, immune cell composition and functionality in blood, spleen, and peritoneum were assessed by flow cytometry.
Principal component analysis of global gene expression of naïve bone-marrow monocytes revealed a sustained deregulation of certain genes after CLP conditions: 75 genes were differentially expressed, with 2 down- and 73 up-regulated genes. Furthermore, an increase of H3K4me3 was observed in 77 promoter regions of post-septic naïve monocytes. No correlation between changes in H3K4me3 and altered gene expression could be determined. Furthermore, a robust change of immune cell abundance, especially of the lymphoid lineage, in spleen and peritoneum was obvious.
Our results prove the remains of transcriptomic scars in naïve bone marrow monocytes even months after the insult, potentially indicating an *ab initio* altered functional state of naïve monocytes. Interestingly, the increase in gene expression was not associated with histone alterations, leaving the question of the involved regulatory tier open for further research.

P.C6.06.04
Ouabain reduces adhesion molecule CD18 expression on neutrophils

L. Cavalcante-Silva[1], *É. Lima*[2], *D. Carvalho*[2], *J. Galvão*[1], *J. Costa*[2], *S. Rodrigues-Mascarenhas*[2];
[1]*Health Science Center, João Pessoa, Brazil,* [2]*Biotechnology Center, João Pessoa, Brazil.*

Introduction: Ouabain, a hormone which inhibits Na^+,K^+-ATPase, is capable to modulate many aspects of the inflammatory process. We have previously demonstrated that ouabain inhibits neutrophil migration in several inflammatory models, but little is known about the mechanisms involved. Thus, the aim of this work was to evaluate ouabain effect on molecules related to neutrophil migration. Materials and Methods: Neutrophils obtained from mice bone marrow (ethical committee number 039/2015), after Percoll gradient separation, were seeded into 96-well plates and treated with ouabain (1 nM, 10 nM and 100 nM) at different times (2, 4 and 24h). Possible ouabain cytotoxicity was assessed by anexin V/propidium iodide staining. Ouabain effect on adhesion molecule CD18 and chemokine receptor CXCR2 was evaluated by flow cytometry. Statistical analysis was performed using ANOVA one way followed by Dunnet test. Results: Ouabain treatment did not reduce neutrophil viability at the periods studied. However, neutrophil basal viability was reduced after 4h, thus we assessed ouabain effect on adhesion molecule CD18 and chemokine receptor CXCR2 only after 2h of treatment. CXCR2 expression on neutrophil membrane was not affected by ouabain treatment (1, 10 and 100 nM). On the other hand, 1 nM ouabain reduced CD18 expression (30%), an integrin beta chain protein. Conclusion: These data suggest that ouabain inhibitory effect on neutrophil migration was related to CD18 reduced expression, suggesting a new mode of action of this substance. Funding support: CNPq (fellowship nº 140856/2016-1) and CAPES/PROCAD (grant nº 2951/2014).

P.C6.06.05
Allele-specific Alternative Splice Transcripts for MHC class I-like MICA encode Novel NKG2D Ligands with Agonist or Antagonist function

B. CHARREAU[1], *P. Gavlovsky*[1], *P. Tonnerre*[1], *N. Gérard*[1], *Y. Hamon*[1], *S. Nedellec*[2], *A. Daman*[3], *B. J. McFarland*[3];
[1]*CRTI, Nantes, France,* [2]*Plateforme MicroPICell SFR Santé –IRT, Nantes, France,* [3]*Department of Chemistry and Biochemistry, Seattle Pacific University, Seattle, United States.*

Major histocompatibility complex (MHC) class I chain-related proteins A and B (MICA and MICB) and UL16 Binding Proteins (ULBPs) are ligands of the activating NKG2D receptor involved in cancer and immune surveillance of infection. Structurally, MICA/B proteins contain a α3 domain, while ULBPs do not. We identified novel alternative splice transcripts (AST) for MICA encoding five novel MICA isoforms: MICA-A, -B1, -B2, -C, and -D. Alternative splicing selectively associates with MICA *015 and MICA*017 alleles and results from a point deletion (G) in the 5' splice donor site of MICA intron 4 leading to exon 3 and exon 4 skipping and/or deletions. These changes delete the α3 domain in all isoforms, and the α2 domain in the majority of isoforms (A, B1, C, and D). Endothelial, hematopoietic and tumor cells from MICA *015 and MICA*017 individuals display endogenous AST and isoforms. MICA-B1, -B2 and -D bound NKG2D by surface plasmon resonance and were expressed at the cell surface. Functionally, MICA-B2 contains two extracellular domains (α1 and α2) and is a novel potent agonist ligand for NKG2D. MICA-D is a new truncated form of MICA able to bind NKG2D despite deletions of α2 and α3 domains that may functionally impair NKG2D activation. In conclusion, truncated MICA isoforms exist and exhibit a range of functions that may drive unexpected immune mechanisms and provide new tools for immunotherapy.

P.C6.06.06

Inflammatory/pro-degradative protein signature in tissues obtained from around aseptically loosened total knee arthroplasty

T. Dyskova[1], *J. Gallo*[2], *S. Zehnalova*[3], *M. Kudelka*[3], *P. Schneiderova*[1], *R. Fillerova*[1], *V. Smotkova-Kraiczova*[1], *E. Kriegova*[1];
[1]*Dept. of Immunology, Palacky University, Olomouc, Czech Republic,* [2]*Dept. of Orthopaedics, Palacky University&University Hospital, Olomouc, Czech Republic,* [3]*Dept. of Computer Science, Faculty of Electrical Engineering and Computer Science, VSB-Technical University, Ostrava, Czech Republic.*

Introduction: Inflammatory responses to biomaterial particles released from the bearing surfaces of joint replacements play a crucial role in the initiation and progression of periprosthetic osteolysis (PPOL). There exist only limited data on the immune protein signature in tissues from aseptically loosened (AL) total knee arthroplasty (TKA) and no information is relevant to pre-osteolytic stages in TKA. Methods: Therefore, we aimed to elucidate the protein signature of 92 inflammation-related proteins using a high-sensitive Proximity Extension Assay (PEA) in pseudosynovial tissue lysates obtained from patients re-operated for PPOL/AL (TKA, n=12) and those with TKA and no clinical and radiographic signs of PPOL/AL (C-TKA, n=9), re-operated for pain or fracture. Results: Late osteolysis was associated with elevated levels of TNF-family members TNFR2, TNFSF14, FasL, and BAFF, interleukins IL-8/CXCL8, IL-1RA/IL-36, sIL-6R, and growth factors AREG and CSF1 comparing to pre-osteolytic stages. Interestingly, analysis of time-axis revealed only weak association of inflammatory/pro-degradative protein signature and time from index surgery in periprosthetic tissues, thus further supporting particle-dose- rather than time-dependent inflammatory processes around TKA. Conclusion: The characterization of inflammatory signature and its dynamic changes during PPOL development may lead to better understanding the osteolytic process and thus proposal of effective preventative/therapeutic strategies contributing to reduction of this most common late complication in TKA.
Grant support: MZ CR VES16-31852A, MZ ČR VES15-27726A, IGA UP_2018_016, MH CZ - DRO (FNOL, 00098892)

P.C6.06.07

Silver nanoparticles induced neutrophil extracellular trap formation: reactive oxygen species-dependent citrullination and cleavage of histones

H. Kang*, J. Seo, I. Choi;*
Department of Microbiology and Immunology, Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine and Institute for Immunology and Immunological Disease, Seoul, Korea, Republic of.

Introduction: Neutrophils are responsible for the first line of defense in innate immune systems. As one of the immune responses, they expel their own DNA to trap and eliminate pathogens known as neutrophil extracellular traps (NETs). However, excessive NET formation causes several autoimmune diseases such as rheumatoid arthritis, lupus erythematosus and atherosclerosis. Silver nanoparticles (AgNPs) have been used in a variety of fields because of their antimicrobial property. However recent studies showed that AgNPs induce reactive oxygen species (ROS) generation which is a key factor for NET formation.
Materials and Methods: Human neutrophils were isolated from healthy donors and seeded in cell culture plates. After that, AgNPs were treated for 4 h. To investigate the mechanism of AgNP-induced NET formation, various inhibitors were pretreated. NETs were quantified using the Picogreen dsDNA quantification assays and visualized by confocal imaging of extracellular DNA and granular components. Citrullination and cleavage of histones were detected by western blot.
Results: 5 nm AgNPs induced NET formation. On the other hand, 100 nm AgNPs not induced NET formation in the same concentration or surface area. N-acetylcystein, ROS scavenger, strongly reduced 5 nm AgNP-induced NET formation. Under the downstream of the ROS, peptidyl arginine deiminase and neutrophil elastase inhibitors reduced 5 nm AgNP-induced NET formation.
Conclusions: In this study, we revealed that 5 nm AgNPs induce NET formation through ROS dependent citrullination and degradation of histones. Based on these results, we are planning to investigate relationship between NET related diseases and AgNPs.

P.C6.06.08

Effects of Quinton solution on human neutrophils' respiratory burst

A. B. López-Jaén[1], *P. Martínez-Peinado*[1], *S. Pascual-García*[1], *G. Peiró-Cabrera*[1,2], *F. J. Navarro-Blasco*[1,3], *J. M. Sempere-Ortells*[1];
[1]*University of Alicante, Alicante, Spain,* [2]*General University Hospital of Alicante, Alicante, Spain,* [3]*General University Hospital of Elche, Elche, Spain.*

Introduction. Quinton® Isotonic Solution consists in ultrafiltered diluted seawater with a final concentration of 9 g/l NaCl, obtained from nutrient-rich areas (marine vortices). The solution contains most of the mineral elements necessary for the correct functioning of the body's cells. In our previous studies, Quinton® isotonic solution has shown different immunomodulatory activities on lymphocytes. Neutrophils phagocytic response generates reactive oxygen species (oxidative burst), with a key role in the defense against pathogens. The aim of this study is to analyse the effect of Quinton® isotonic solution on neutrophils function. Materials and Methods. Human neutrophils were isolated by density gradient centrifugation with Percoll, from anticoagulated blood samples of healthy donors. 100.000 neutrophils were cultured in rounded 96-well plates, with different ratios of RPMI and Quinton® isotonic solution (100%, 50%, 25%, 12,5%, 0%) for 12 hours, in a final volume of 200 uL. PBS solution was used as a control. Respiratory burst was induced by incubating cells with phorbol 12-myristate 13-acetate (PMA) (0,3 ng/mL) for 30 minutes at 37 ºC, 5% CO_2, and measured by flow cytometry after adding dihydrorhodamine 123 (DHR). Results. Quinton® isotonic solution increased the percentage of positive neutrophils for respiratory burst, with and without stimulation. In the case of stimulated cultures, the increase was significant for the 50% concentration, with nearly significant p-value for the 25% concentration. Conclusion. Quinton® isotonic solution, under proper concentrations, seems to modulate neutrophils' function in vitro through an increase in their respiratory burst, which could translate into a higher bactericidal activity.

P.C6.06.09

Experimental mouse Graft-versus-Host-Disease (GvHD) is ameliorated by mesenchymal stem cell derived extracellular vesicle treatment and might involve innate immune activity

R. J. Madel[1], *C. Chebrolu*[1], *V. Börger*[2], *P. A. Horn*[2], *H. A. Baba*[3], *D. Beelen*[4], *L. Kordelas*[4], *A. Bleich*[5], *J. Buer*[1], *B. Giebel*[2], *C. J. Kirschning*[1];
[1]*Institute of Medical Microbiology, University Hospital Essen, University Duisburg-Essen, Essen, Germany,* [2]*Institute of Transfusion Medicine, University Hospital Essen, University Duisburg-Essen, Essen, Germany,* [3]*Institute of Pathology, University Hospital Essen, University Duisburg-Essen, Essen, Germany,* [4]*Clinic of Bonemarrow Transplantation, University Hospital Essen, University Duisburg-Essen, Essen, Germany,* [5]*Laboratory Animal Science, Hannover Medical School, Hannover, Germany.*

Graft-versus-host disease (GvHD) is driven by an often-fatal immune reactivity to allogeneic bone marrow transplantation (BMT). In a previous, unique, and curative treatment at the local Hospital, mesenchymal stem cell derived extracellular vesicles (MSC-EVs) were administered to a steroid-refractory acute GvHD-patient, which persistently ameliorated pathology. BMT is preceded by preconditioning, namely self bone marrow ablation by chemotherapy or ionizing irradiation (IIR) to channel comprehensive graft stem cell homing. Preconditioning-driven tissue damage (TD) might collaterally promote formation of an inflammatory milieu, which might abet GvHD pathogenesis. MSC-EV validation and mechanistic analysis *in vivo* and *-vitro* is our program.
Given TD sensing's conceptual dependence upon innate immune activity, interference with pattern recognition receptor (PRR) activity - possibly encompassed in immune inhibitory MSC-EV function - might antagonize pathology. Experimentally modeling IIR-driven tissue impacting, we observed immune reactivity over an extended period of time *in vivo* and *-vitro*, which was rapid, largely independent upon bacterial colonization, and potentially inhibitable by MSC-EVs. Unexpectedly, BMT *Myd88⁄* mice succumbed to an early pathology, as if TLR/IL-1-type cytokine dependent activity might contribute to health maintenance rather than an adversativeness upon BMT. Notably, we observed in major histocompatibility mismatch BMT driven GvHD-mice a substantial attenuation of pathology upon MSC-EV administration at 3 consecutive days upon BMT. To narrow down a mechanistic cause, comparative analysis of distinct MSC-EV batches and other EVs as controls is being performed *in vivo* and *-vitro*. Our results imply a potential of MSC-EVs to interfere with GvHD pathology at different phases.
Funded by SEVRITLeitmarktLifeScience.NRW LS-1-1-051g

P.C6.06.10

Innate signaling in central nervous system recruits myeloid suppressor cells

J. Marczynska[1], *R. M. Khorooshi*[1], *R. Storgaard Dieu*[1], *C. Rønn Hansen*[2], *S. Kavan*[3], *M. Thomassen*[3], *M. Burton*[3], *T. Kruse*[3], *G. A. Webster*[4], *T. Owens*[1];
[1]*Department of Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark,* [2]*Laboratory of Radiation Physics, Odense University Hospital, Denmark. Institute of Clinical Research, University of Southern Denmark, Odense, Denmark,* [3]*Unit of Human Genetics, Department of Clinical Research, University of Southern Denmark, Odense, Denmark,* [4]*Innate Immunotherapeutics, Auckland, New Zealand.*

Introduction: Regulation of neuroinflammation is necessary to maintain central nervous system (CNS) homeostasis and has therapeutic potential in diseases such as multiple sclerosis. The CNS contains parenchymal and extraparenchymal myeloid cells that can play a regulatory role. We have examined the capacity of innate-signaled myeloid cells to regulate experimental autoimmune encephalomyelitis (EAE) in mice.
Methods: Mice received a fluorescent-conjugated bispecific NOD2- and TLR9-agonist microparticle MIS416 either intravenously or by intrathecal injection to extraparenchymal leptomeningeal, ventricular and subarachnoid space. MIS416 has previously been shown to suppress EAE when given peripherally.
Results: Intravenous MIS416 induced significant extraparenchymal infiltration from blood of monocytic myeloid cells ($CD45^{high}$, Ly6C+, F4/80+, CD11b+, CD11c+) that had phagocytosed MIS416. Intrathecal MIS416 induced infiltration of similar magnitude but in contrast to peripheral injection, over 30% of the MIS416-phagocytosing cells were granulocytic ($Ly6G^{high}$, $Ly\text{-}6C^{low}$, $Gr1^{high}$, CD11b+, CD11c-). Both populations were also PDL1+. mRNA for the neutrophil-recruiting chemokines CXCL1 and CXCL2 were upregulated in CNS.
When given to mice showing first symptoms of EAE, intrathecal MIS416 suppressed disease - this did not occur in mice lacking the Type I IFN receptor. Extraparenchymal MIS416-phagocytosing cells, including polymorphonuclear neutrophils, were shown to produce IFNβ in reporter mice, and sorted monocytic and granulocytic cells expressed IRF7, indicating IFN response.
Conclusions: CNS-innate signaling uniquely recruits granulocytic myeloid-derived phagocytes from blood. These and co-infiltrating phagocytic monocytic myeloid cells produce IFNβ and suppress EAE by a Type I IFN-dependent mechanism. This endogenously-triggered pathway likely contributes to CNS homeostasis and may have therapeutic potential.

P.C6.06.11

Analysis of C-C chemokine receptor like 2 (CCRL2) heterodimerization with classical chemokine receptors

C. Mazzotti[1], *A. Bani*[1], *M. Mellado*[2], *A. Del Prete*[1,3], *S. Sozzani*[1];
[1]*University of Brescia, Brescia, Italy,* [2]*Centro Nacional de Biotecnologia, Madrid, Spain,* [3]*Humanitas Clinical and Research Centre, Rozzano, Italy.*

Introduction: Atypical chemokine receptors (ACKRs) are 7-transmembrane receptors able to bind and scavenge chemokines from the local environment. We recently published that the non-signalling receptor C-C chemokine receptor-like 2 (CCRL2), homologous to ACKRs, is devoid of scavenging ability, but it can heterodimerize and regulate the function of the chemokine receptor CXCR2 (Mazzotti et al Front Immunol 2017; Del Prete et al Blood, 2017). The present work aims to further investigate the ability of CCRL2 to regulate additional chemokine receptors.
Materials and Methods: Förster Resonance Energy Transfer (FRET) was employed to measure heterodimerization, using saturation curves and acceptor photobleaching. Measurement of mouse neutrophil adhesion under shear stress conditions was performed to evaluate functional interactions.
Results: Both the FRET saturation curves and acceptor photobleaching showed that CCRL2 can heterodimerize with two chemokine receptors, namely CXCR4 and CCR7. To evaluate the biological significance of these interactions, neutrophil adhesion under shear stress conditions was tested on channels coated with CXCL12, the CXCR4 ligand. Neutrophils lacking the expression of CCRL2 showed altered adhesion compared to wild-type cells.
Conclusions: CCRL2 lacks both signalling and scavenging ability. Our previous publications demonstrated that CCRL2 can regulate CXCR2 activity by heterodimerization. This work shows that CCRL2 can heterodimerize with the classical chemokine receptors CXCR4 and CCR7. The lack of CCRL2 modifies the response of CXCR4-expressing neutrophils to the ligand CXCL12. These results propose that CCRL2 heterodimerization may be a general mechanism to fine tune the biological action of chemokine receptors.
The Presenter was awarded a EFIS-IL Short-term Fellowship (2017)

P.C6.06.12

Minor immunomodulatory role of reactive oxygen species and nitrogen species (ROS/RNS) during T-cell driven neutrophil-enriched acute and chronic cutaneous delayed-type hypersensitivity reaction (DTHR)

R. Mehling[1], *J. Schwenck*[1,2], *B. Zhou*[1], *D. Hartl*[3,4], *M. Röcken*[5], *B. Pichler*[1], *M. Kneilling*[1,5];
[1]*Werner Siemens Imaging Center, Eberhard Karls University, Tübingen, Germany,* [2]*Department of Nuclear Medicine, Eberhard Karls University, Tübingen, Germany,* [3]*Department of Pediatrics I, Eberhard Karls University, Tübingen, Germany,* [4]*Discovery and Translational Area, Roche Pharma Research & Early Development, Basel, Switzerland,* [5]*Department of Dermatology, Eberhard Karls University, Tübingen, Germany.*

ROS and RNS are important regulators of inflammation. However, the impact of ROS/RNS mainly produced by neutrophils and macrophages during acute and chronic DTHR of the skin is discussed controversy. Our aim was to dissect the dominant ROS/RNS sources non-invasively in vivo using the ROS/RNS-sensitive chemiluminescence optical imaging probe L-012 in mice with differently impaired ROS/RNS (Myeloperoxidase ($MPO^{-/-}$), NADPH oxidase ($gp91^{phox-/-}$), inducible nitric oxide synthase ($iNOS^{-/-}$)) production during the acute and chronic trinitrochlorobenzene induced cutaneous DTHR.
Non-invasive *in vivo* optical imaging measurements revealed an abrogated L-012 signal intensity in ears of $gp91^{phox-/-}$ mice and an up to 70% decreased L-012 signal intensity in ears of $MPO^{-/-}$ compared to WT mice during acute and chronic cutaneous DTHR. In contrast, inflamed ears of $iNOS^{-/-}$ mice exhibited L-012 signal intensity similar to WT mice. Dihydrorhodamin 123 flow cytometry analysis of leucocytes derived from the spleen and the draining lymph nodes of knock-out and WT mice confirmed our optical imaging results. However, ROS/RNS depletion did not influence inflammatory symptoms as we observed almost equivalent ear-swelling responses in knock-out and the WT mice during acute and chronic DTHR. Also suppression of mitochondrial ROS in $gp91^{phox-/-}$ mice with MitoTEMPO revealed no relevant influence.
Collectively, we were able to identify MPO-mediated hypochlorous acid (HOCl) production and superoxide anion (O_2^-) produced by NADPH oxidase as the dominant sources for ROS in our model of DTHR. Nevertheless, ROS/RNS expression by neutrophils and macrophages seem to play a rather minor role in the modulation of acute and chronic cutaneous DTHR.

P.C6.06.13

Chemotactic, phagocytic and neutrophil extracellular trap (NET) forming properties of oral and circulatory blood neutrophils

C. G. J. Moonen[1], *J. Hirschfeld*[2], *L. Cheng*[1], *I. L. Chapple*[2], *B. G. Loos*[1], *E. A. Nicu*[1,3];
[1]*Academic Center of Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, Amsterdam, Netherlands,* [2]*Birmingham Dental School and Hospital, Institute of Clinical Sciences, The University of Birmingham and Birmingham Community Health NHS Trust, Birmingham, United Kingdom,* [3]*Opris Dent SRL, Sibiu, Romania.*

Maintenance of oral health is, in part, managed by the immune-surveillance and antimicrobial functions of neutrophilic polymorphonuclear leukocytes (PMNs) that have migrated from the blood circulation (cPMN) through the oral mucosal tissues as oral PMNs (oPMNs). In any microorganism rich ecosystem, such as the oral cavity, PMNs migrate towards exogenous chemoattractants, phagocytose bacteria and produce NETs to immobilize and eliminate pathogens. cPMNs have been widely studied *ex-vivo* using various functional assays. We hypothesized that oPMNs offer a more appropriate model to study the role of PMNs in maintaining oral health.
oPMNs and cPMNs were isolated from healthy donors. Directional chemotaxis towards the chemoattractant fMLP was analysed using an Insall chamber and video microscopy. Phagocytosis was analysed by flow cytometry based on CD16+FITC+ gating of PMNs incubated with heat-inactivated FITC-labelled *Fusobacterium nucleatum (Fn*). NET formation by oPMNs and cPMNs was quantified fluorometrically using Sytox Green after stimulation with either PMA or RPMI medium (unstimulated control).
In contrast to cPMNs, chemotactic responses of oPMNs towards fMLP did not differ from unstimulated controls. oPMNs show reduced speed, velocity and directional movement towards fMLP when compared to cPMNs, which could be explained by exhausted chemotaxis capacity after having migrated through oral tissues into the oral cavity, being a highly 'hostile' ecosystem. oPMNs and bPMNs phagocytosed *Fn* similarly. Unstimulated and stimulated oPMNs formed significantly more NETs than cPMNs. Based on observed normal phagocytosis but hyperreactive NET production by unstimulated oPMNs, we conclude that oPMNs are primed due to their exposure to oral bacteria.

P.C6.06.14

Neutrophil extracellular traps in type 1 diabetes

Z. Parackova[1], *I. Zentsova*[1], *Z. Sumnik*[2], *S. Kolouskova*[2], *A. Klocperk*[1], *P. Vrabcova*[1], *A. Sediva*[1];
[1]*Department of Immunology, 2nd Faculty of Medicine Charles University, Faculty Hospital in Motol, Prague 5, Czech Republic,* [2]*Department of Pediatrics, 2nd Faculty of Medicine Charles University, Faculty Hospital in Motol, Prague 5, Czech Republic.*

Neutrophil extracellular traps (NETs) were demonstrated to be an effective defence mechanism against infections, being able to entrap and eliminate various pathogens. These structures, composed of decondensed chromatin and antimicrobial proteins, also have the capacity to stimulate other cell subsets, for instance macrophages and dendritic cells (DC). Additionally, NETs are implicates in several autoimmune diseases, such as lupus erythematodes, vasculitides or type 1 diabetes (T1D) but their contribution to pathogenesis is elusive.In T1D, insulin-producing pancreatic beta cells are destroyed by autoreactive T lymphocytes. Even though innate immunity involvement is also clearly demonstrated in the development of T1D, its exact mechanism still remains unclear. To examine how neutrophils contribute to the genesis of T1D, we investigated the effect of NETs on DC function in T1D patients. We found that NETs influence mDC phenotype differently between patients and controls, but not pDC phenotype. They also triggered T1D DCs to release inflammatory cytokines - IFNα and IL-1β.
On the contrary, T1D patient DCs primed with NETs (NET-DCs) produced lower level of IL-10. NET-DCs induced more IFNγ-producing CD4+ T lymphocytes and less T regulatory lymphocytes (Tregs) compared to controls. In addition, patients NET-DCs-induced Tregs expressed a low level of IL-2Rα, which implies their impaired function. Moreover, T1D-NETs contain more DNA and less antimicriobial peptides. So not only NETs differ between T1D and controls in effect on DCs, but also in their composition. Our findings shed light on the involvement of dendritic cells in pathogenesis of T1D, through their interaction with neutrophils.

P.C6.06.15

Redefining the functional site of the innate immune regulator 'complement Factor H'

C. Q. Schmidt, *A. Dopler, L. Guntau;*
Institute of Pharmacology of Natural Products & Clinical Pharmacology, Ulm University, Ulm, Germany.

Factor H (FH) is the major regulator of the complement alternative pathway (AP) and is built of 20 CCP domains. FH crucially controls the AP (i) with 'decay accelerating activity' (DAA) towards C3-convertases and (ii) with cofactor activity (CA) for the inactivation/degradation of the opsonin C3b which e.g. initiates C3-convertases. To achieve DAA and CA, FH needs to interact with C3b. It is established that the C3b binding activity necessary for these two functions resides within the first four N-terminal domains of FH, i.e. FH1-4. We prove that this N-terminal regulatory site extends CCPs 1-4 and spans across the first seven domains of FH.
We recombinantly expressed the FH domain constructs FH1-4, FH1-5, FH1-6 and FH1-7 and probed their different regulatory activities in following *in vitro* tests: determination of C3b affinity by surface plasmon resonance (SPR), fluid phase CA assay, SPR-based DAA assay and a protection assay of microvascular endothelial cells.
While FH1-5 binds only marginally better to C3b than FH1-4, we show that FH1-6 and FH1-7 surpass the affinity of FH1-4 for C3b about threefold and 15-fold, respectively. Despite the small difference in C3b affinity, FH1-5 substantially surpassed FH1-4 in DAA and CA. Both, FH1-6 and FH1-7, were substantially more active in DAA and CA than the shorter constructs tested. Consistent with these rankings FH1-6 and FH1-7 protected microvascular endothelial cells considerably better from complement attack than FH1-4 or FH1-5 did. Taken together, our study extends the established N-terminal regulatory site of FH1-4 by CCPs 5-7 (DFG-grant: SCHM 3018/2-2).

P.C6.06.16

The effect of catecholamines on spontaneous and lipopolysaccharide-induced production proinflammatory cytokines by human neutrophils *in vitro*

I. Shvydchenko[1], *E. Bykovskaya*[2], *S. Guryanova*[3], *V. Golubtsov*[4,5], *A. Tambovtseva*[5,1], *S. Sergeev*[1];
[1]*Kuban State University of Physical Education, Sport and Tourism, Krasnodar, Russian Federation,* [2]*Children's Regional Clinical Hospital, Krasnodar, Russian Federation,* [3]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russian Federation,* [4]*Kuban State Medical University, Krasnodar, Russian Federation,* [5]*Regional Clinical Hospital №2, Krasnodar, Russian Federation.*

Neutrophils are essential for innate immunity and resistance to infections. Adrenergic pathways represent the main channel of communication between the nervous system and the immune system. The purpose of this study was to investigate the effect of adrenaline and noradrenaline on cytokine production by human neutrophils *in vitro*. Neutrophils were isolated by two methods, as follows: by density gradient centrifugation of whole blood, dextran sedimentation and red cells osmotic lysis (*Neu1*); similarly to *Neu1*, but with an additional immunomagnetic negative selection step, using the EasySep Human Neutrophil Enrichment Kit (*Neu2*). According to morphological analysis and flow cytometry 92-98% pure neutrophils were in *Neu1* and > 99% was in *Neu2*. Neutrophils (1×10^6 cells/ml) were incubated in medium RPMI 1640 (37°C, 5% CO_2, 20 h) with DPBS or 10 ng/ml lipopolysaccharide (LPS), adrenaline (0,1 μM), noradrenaline (0,1 μM). IL-1β, IL-6, IL-8, TNF-α and MIP-1β in cell-free supernatants were measured by ELISA. IL-8 and MIP-1β, but not IL-1β, IL-6 and TNF-α were detected in supernatants from non-stimulated neutrophils in *Neu1* and *Neu2.* Secretion of IL-1β, IL-8 and MIP-1β by neutrophils in *Neu1* and *Neu2* was increased with LPS ($p<0.05$). Adrenaline did not affect on spontaneous and LPS-induced secretion of all cytokines by neutrophils in *Neu1* and *Neu2.* Noradrenaline increased production only of IL-8 by non-stimulated neutrophils in *Neu1* ($p<0.05$). Taken together, our results indicate that catecholamines have the limited role in secretion of proinflammatory cytokines by neutrophils. This work was supported by a grant from RFBR and Administration of Krasnodar Region (No 16-44-230391r_a).

P.C6.06.17

Inhibition of dendritic cell maturation and modulation of T cell polarization by the platelet secretome

J. Steuten[1], *A. S. Saris*[1], *S. M. van Ham*[1,2], *A. ten Brinke*[1];
[1]*Department of Immunopathology, Sanquin Research, Amsterdam, Netherlands,* [2]*University of Amsterdam, Swammerdam Institute for Life Sciences, Amsterdam, Netherlands.*

Next to their primary function in hemostasis, currently there is increased attention for the immune modulatory capacity of platelets and how they are able to influence various cells of the immune system. We have found that platelets can inhibit pro-inflammatory properties of TLR-stimulated monocyte-derived dendritic cells (moDCs), as well as their capacity to prime both autologous and allogeneic T cells. Here, we further investigate the various immune modulatory capacities of platelets with respect to dendritic cells and the subsequent effect on T cell responses. First of all, by culturing moDCs in the presence of different maturation cocktails, we obtained moDCs with the capacity to induce different T helper phenotypes. Subsequently, we investigated the effect of platelets and platelet-conditioned medium on the moDCs themselves as well as ensuing T helper differentiation. Here we demonstrate that platelets can significantly inhibit pro-inflammatory properties of moDCs and modify subsequent T cell priming and differentiation of T helper cell phenotypes. Secondly, transwell assays revealed that a soluble factor is secreted by platelets which exerts the inhibitory effect on moDC maturation. Therefore, we are currently fractionating platelet-conditioned medium to identify which component in the platelet secretome is responsible for this immune modulatory effect of platelets. The results of these assays will eventually give us insight in the role of platelets in for example transfusion related immune modulation (TRIM) in the context of platelet concentrate transfusions.

P.C6.06.18

Systemic pDC death causes type I interferon-mediated reduction of appetite, fatal weight loss and immune cell attrition

S. Stutte[1], *J. Ruf*[1], *I. Kugler*[1], *H. Ishikawa-Ankerhold*[2], *A. Blutke*[3], *P. Marconi*[4], *U. von Andrian*[5], *M. Colonna*[6], *S. Massberg*[2], *T. Brocker*[1];
[1]*Institute of Immunology, LMU, Munich, Germany,* [2]*Medizinische Klinik und Poliklinik I, Ludwig-Maximilians-Universität, Munich, Germany,* [3]*Institute of Veterinary Pathology at the Centre for Clinical Veterinary Medicine, Ludwig- Maximilians-Universität Munich, Munich, Germany,* [4]*Department of Life Science and Biotechnology, University of Ferrara, Ferrara, Italy,* [5]*Microbiology and Immunobiology, Harvard Medical School, Boston, United States,* [6]*Washington University, School of Medicine, St. Louis, United States.*

Plasmacytoid dendritic cells (pDCs) develop in the bone marrow and are constantly released into the blood circulation from where they home to peripheral organs. pDCs belong to the innate immune cell compartment and are known to rapidly release very high amounts of type one interferon in response to viral infections. In addition, thereafter pDC die by apoptosis. Here we analyzed the consequence of pDC-death under "sterile" conditions (in absence of viral infection) using ablation of pDCs in a diphtheria toxin-mediated in vivo ablation model. We could show that loss of pDCs mediates systemic changes, resulting in reduced appetite, strong weight loss and fatal wasting disease. In addition, we observed an attrition of immune cells in lymphoid organs. We identified the innate signaling pathways leading to type I interferon production, which mediates alteration of key soluble factors central to these rapid changes and analyzed consequences for ongoing immune responses. Taken together, death of pDCs causes dramatic systemic changes in metabolism and immune cell homeostasis.

P.C6.06.19

Increased IL-22 levels in relapsing-remitting Multiple Sclerosis

I. Tahrali[1], *U. C. Kucuksezer*[1], *N. Akdeniz*[1], *A. Altintas*[2], *U. Uygunoglu*[2], *E. Cetin*[1], *G. Deniz*[1];
[1]*Aziz Sancar Institute of Experimental Medicine, Istanbul, Turkey,* [2]*Istanbul University, Cerrahpasa Faculty of Medicine, Istanbul, Turkey.*

IL-22 is a proinflammatory cytokine produced by different cell groups including NK22 cells. Several studies suggested that IL-22 might play a critical role in neurological diseases such as multiple sclerosis (MS), a chronic-inflammatory disease of central nervous system. However, the exact role of IL-22 in MS pathology is still a question. The possible role of IL-22 in MS patients was investigated in this study.
Plasma samples and peripheral blood mononuclear cells (PBMCs) were obtained from patients with relapsing-remitting (RR)-MS (untreated and treated with IFN-beta) and clinically isolated syndrome (CIS) as well as healthy subjects.
PBMCs were cultured with/without hrIL-2 and hrIL-12. IL-22 levels in plasma and culture supernatants were measured by ELISA. Cultured PBMCs were stained with anti-CD3, -CD16 and -CD56 monoclonal antibodies (mAb) to detect NK cell subsets by flow cytometry. Stained cells were fixed/permeabilized and labeled with anti-IL-22 mAb to evaluate the intracellular IL-22 contents of NK cell subsets.
After hrIL-2 stimulation, IL-22 content of $CD3^-CD16^-CD56^{bright}$ NK cell subset was increased in treated RR-MS patients compared to healthy subjects. In contrast, plasma IL-22 levels were higher in untreated RR-MS patients than CIS patients. IL-22 levels were also increased in unstimulated and hrIL-2 stimulated supernatants of untreated RR-MS patients compared to treated RR-MS patients and healthy subjects.
Increased IL-22 levels in plasma samples and supernatants of untreated RR-MS patients might indicate the proinflammatory role of IL-22 in RR-MS pathogenesis.

P.D1.01 Microbiome, metabolites and the immune system - Part 1

P.D1.01.01

Study Of Toll-Like Receptor 7 (TLR7) Expression In Iraqi Patients With Chronic Hepatitis C Virus (HCV) Infection & their relationship with different genotypes

E. S. Al0beidy;
Ministry of Health, Baghdad, Iraq.

Introduction: The main etiologic agent of post-transfusion hepatitis is Hepatitis C (HCV) and is considered the principal cause of chronic liver disease in multi-transfused patients. TLR7 on the other hands, play a significant role in the viral hepatitis infection.
This study was established to estimate the concentration of TLR7 in patients with chronic HCV infection compared to healthy control group.
Patients and methods: Throughout the period from December 2016 to March 2017, a blood sample from 40 patients (17 male and 23 females) with chronic HCV infection and 16 healthy control group (7 male and 9 females) were collected from two hospitals in Baghdad city.
All serum samples (n=56) were screened for presence of HCV antibodies using the available commercial kits of the third generation enzyme immunoassay (Human-Gemeny).and for TLR7 by ELISA method (Shanghai-China).
Results: genotype 1b is most common one since they represent (52.5%)compared with other allele 1a,2,4 %),((22.5%), (5.0%), (20.0%) respectively. In addition, the current study noted a significant decrease in the TLR7 concentration in sera of patients affected with HCV compared with healthy control. In addition, our study revealed that there is no statistical differences in the age and gender between the studied groups. On the other hands, the mean concentration of TLR7 was decreased in HCV group in comparison with healthy control.
Conclusions:
The predominance of HCV genotype 1b in our population confirms the predominance of HCV genotype 1b in Iraqi and most of the Arab countries in the Middle East, this will effect the duration and response to treatment.

P.D1.01.02

Capture of IgA immune complexes and enrichment in IgA Ig gene expression both suggest a role for synovial FcRL4+B cells in the link between mucosal and joint inflammation

J. Cameron[1], *E. Clay*[1], *K. Amara*[2], *N. Sippl*[2], *A. Filer*[1], *K. Raza*[1], *V. Malmstrom*[2], *D. Scheel-Toellner*[1];
[1]*Institute of Inflammation and Ageing, Birmingham, United Kingdom,* [2]*Karolinska Institutet, Stockholm, Sweden.*

Mounting evidence points to the autoimmune process of rheumatoid arthritis (RA) originating at mucosal surfaces. FcRL4$^+$ B cells were originally identified in the mucosa associated lymphoid tissue. We recently detected and characterised these cells in the inflamed joints of patients with RA. They participate in the immune response to citrullinated autoantigens and produce RANKL, a cytokine driving bone destruction in RA. Recent *in vitro* work suggested that FcRL4 is a low affinity receptor for heat-aggregated IgA (HA-IgA). We explored the link between joint derived FcRL4+ B cells and mucosal immunity. We looked at the interaction between FCRL4+ B cells and IgA in RA synovial fluid (SF) and investigated the distribution of Ig subclasses via flow cytometry and PCR of constant region genes of single sorted B cells; and using antibodies cloned from their variable regions we assessed the antigen specificity of FcRL4+ and FcRL4- cells. SF FcRL4$^+$ B cells have a significantly higher load of surface-bound IgA than FcRL4$^-$ B cells. Following removal of surface-bound proteins, FcRL4+ B cells bound heat-aggregated IgA. A significantly higher proportion of FcRL4$^+$ B cells use IgA B cell receptors (BCRs). B cells transgenic for FcRL4 expression captured IgA immune complexes from SF and three out of eight of the antibodies recognizing citrullinated peptides were cloned from FcRL4$^+$ IgA$^+$ B cells. In conclusion, their specificity for citrullinated antigens, ability to capture IgA immune complexes in vivo, and enrichment in IgA BCRs support a role for FcRL4+ B cells in the mucosal origin of joint inflammation.

P.D1.01.03

The role of gut microbiome in the pathogenesis and therapeutic response in juvenile idiopathic arthritis

C. Changchien*, B. Chiang;*
National Taiwan University Hospital, Taipei, Taiwan.

Introduction: Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in children. However, the pathogenesis of JIA was still unclear. From previous animal study, gut microbiome played an important role in host immune systemic development, and defect in mucosal tolerance might attribute to chronic inflammation disease. The aim of the study is to clarify the therapeutic effects on gut microbome in JIA children. **Method**: We collected the samples from sibling healthy control and compare the gut microbiome between sibling healthy control, healthy control and JIA children. Children at the age between 5-17 year-old with JIA under anti-TNF treatment without any antibiotics in past 6 months or recent GI symptoms were enrolled in our study. DNA purified from stool was subject to PCR amplification and sequencing of the variable V3-V4 region from the 16S rDNA gene. The study protocol was approved by the Institutional Review Board of National Taiwan University Hospital (201411083RIN). **Result**: Twenty eight JIA patients and 20 healthy controls were enrolled to our study. The beta-diversity result showed the distribution of gut microbiome in JIA under TNF control was more similar to treatment naïve JIA patients' gut microbiome than JIA under DMARDs control. The observed number of OTUs and the Chao1 index of alpha diversity had significant reduction in JIA patients under TNF therapy compared to JIA patients under DMARDs therapy.**Conclusion**: The results suggested that treatment and the distribution of gut microbiome were highly related. Treatment could also partially restored dysbiosis status in JIA children. <!--EndFragment-->

P.D1.01.04

Interplay between Zika Virus and Decidual Natural Killer cells at the human maternal-fetal interface

Q. CHEN*, J. Gouilly, H. El Costa, N. Jabrane-Ferrat;*
Centre de Physiopathologie de Toulouse Purpan (CPTP), Toulouse, France.

BackgroundThe recent Zika virus (ZIKV) outbreak revealed unprecedented severe adverse pregnancy outcomes including microcephaly and diseases associated to placental dysfunctions. We recently provided evidence for ZIKV productive infection in the first trimester decidua basalis, one of the main maternal-fetal interfaces, and in fetal placenta. We also demonstrated that several cell types such as fibroblasts and macrophages from the maternal-fetal interface, fetal trophoblasts and Hofbauer cells as well as mesenchymal stem cells of the Warton jelly. The hallmark of the decidua basalis is the presence of a unique subset of Natural Killer (dNK) cells. In healthy pregnancy, these cells are devoid of cytotoxicity but they produce several soluble factors that are crucial for fetal tolerance and placental development. Our pioneer work demonstrated that dNK cells can adapt their effector functions and acquire cytotoxic function to protect the fetus from congenital Cytomegalovirus infection. Methodology & resultsWe show here that dNK cells can control ZIKV replication in the decidual stroma. Using double-chamber co-cultures, we demonstrate that the inhibition of ZIKV replication is mediated through the release of soluble mediators by dNK cells. However, changes in the local secretome following ZIKV infection may also modify dNK cell functions and impact their ability to effectively supervise the course of pregnancy. We are currently deciphering the cellular and molecular mechanisms underlying the control of ZIKV infection by dNK cells and the consequences of such control on the outcome of pregnancy.

P.D1.01.05

Intestinal bacteria modulate the anti-inflammatory properties of filarial helminth product ES-62 in arthritis

J. Doonan[1], *A. Tarafdar*[2], *F. Lumb*[1], *J. Crowe*[2], *A. Khan*[2], *M. Pineda*[2], *P. Hoskisson*[1], *M. Harnett*[2], *W. Harnett*[1];
[1]*University of Strathclyde, Glasgow, United Kingdom,* [2]*University of Glasgow, Glasgow, United Kingdom.*

The Hygiene Hypothesis suggests that increased sanitation and decreased exposure to parasitic infections has resulted in aberrant immune responses that have given rise to autoimmune diseases, such as rheumatoid arthritis (RA). Parasitic helminth excretory-secretory products modulate the host's immune system and one such anti-inflammatory product is ES-62, a glycoprotein produced by the rodent filarial helminth *Acanthocheilonema viteae.* We aimed to address the role that intestinal bacteria exert on the anti-inflammatory properties of ES-62 in the collagen-induced arthritis (CIA) model of RA.
Antibiotic treatment (ABX) was used to eliminate intestinal bacteria in DBA/1 mice prior to CIA induction and ES-62 treatment. Histology, qPCR and FACS were used to address the effects of ES-62 and/or dysbiosis on arthritis in the periphery and gut. Metagenomic sequencing was employed to identify intestinal bacteria associated with arthritis or ES-62. Intestinal bacteria were found to be required for the development of arthritis in control animals and CIA was associated with changes in the microbiome. Strikingly, ES-62 required the presence of bacteria to prevent CIA and ES-62 treatment led to a normalisation of the gut microbiome to a naïve phenotype in addition to an outgrowth in butyrate-producing bacteria. In the periphery, ES-62 prevented joint pathology and also reduced Cathepsin K$^+$ osteoclast numbers in joints and stably rewired bone marrow progenitors to inhibit osteoclast differentiation compared to controls.
In summary, ES-62 is associated with normalisation of the gut microbiome and promotion of butyrate-producing bacteria to reduce inflammatory and pathological changes in the joints and gut during CIA.

P.D1.01.06

Modelling the effect of social isolation on susceptibility to sepsis: a murine model

A. Hamilton[1], *S. Brod*[1], *R. Rizzo*[1], *J. Dali*[1], *F. Marelli-Berg*[1], *F. D'Acquisto*[1,2];
[1]*William Harvey Research Institute, Queen Mary University of London, London, United Kingdom,* [2]*Health Science Research Centre, University of Roehampton, London, United Kingdom.*

A person's social network size and quality of life can affect the body at three levels: behavioural, psychological and physiological. Indeed, a socially isolated person is more likely to have a poor diet, suffer from depression and have an impaired immune system. A growing body of evidence has shown that social status correlates with susceptibility to sepsis-mediated death. The aim of this study was to investigate how social isolation effects sepsis and inflammation.
CD1 mice (6 weeks old) were assigned to cages of 4 or 5 mice, socially housed (SH), or singly housed, socially isolated (SI). After 2 weeks, mice were challenged with 1x10^{7}cfu *E.coli* 06:K2:H1[ATCC®19138™] via i.p injection to model bacterial sepsis. Weight loss, bacterial clearance and immune response were assessed. Microarray gene screening of basal whole blood was done to further elucidate changes in gene expression.
E.coli challenged SI mice exhibited enhanced bacterial clearance (~90%) compared to SH mice and lost less weight over the 6 hour period. SI mice also showed decreased levels of TNF-α (~50%) and IL-6 (~80%) systemically compared to SH mice and had increased macrophages present in the peritoneal cavity. Microarray analysis revealed changes in gene expression that promote apoptosis.
In summary, short-term social isolation appears to prime the immune response towards bacterial clearance. Further studies are required to fully elucidate the mechanism(s) by which social isolation impacts the immune system.

P.D1.01.07

Modulation of intestinal homeostasis and inflammation by *Prevotella intestinalis (nov. sp.)*

***A. Iljazovic**, U. Roy, E. Gálvez, B. Zhao, T. Strowig;*
Helmholtz Centre for Infection Research, Braunschweig, Germany.

Prevotella is a complex genus of anaerobic Gram-negative bacteria of the Bacteroidetes phylum. Several studies have suggested *Prevotella copri* may be a beneficial member of the gut microbiota since it has been found to improve glucose metabolism and it is predominantly prevalent in non-Westerners who consume a plant-rich diet. In mouse models, *Prevotella*-dominated microbiome was associated with higher susceptibility to chemically-induced colitis suggesting that *Prevotella* may have the ability to promote intestinal inflammation. Detailed investigation of the cause for divergent modulation of host physiology by *Prevotella* is however limited by the poor characterization of *Prevotella* species and the lack of diverse intestinal *Prevotella* isolates. Here we isolated a novel intestinal *Prevotella* specie (*Prevotella intestinalis)* and investigated the impact of its colonization on the interplay between host and the microbiota during intestinal homeostasis and inflammation. We found that *P. intestinalis* colonization of WT specific pathogen free (SPF) mice, devoid of any *Prevotella spp.* in the intestine, reshapes the resident intestinal microbial community and it significantly alters the metabolic profile in the intestine. *Prevotella*-induced changes in the levels of short-chain fatty acids (SCFA) modulated colonic interleukin (IL)-18 expression and production during homeostasis. Additionally, we found that *P. intestinalis* colonization of WT SPF mice exacerbated the disease in DSS-colitis model and promoted neutrophil-mediated intestinal inflammation. We are further investigating whether *Prevotella*-induced changes occurring during the homeostasis are directly linked to a more severe outcome during inflammation.

P.D1.01.08

Oral metronidazole has immediate microbiota-independent immunosuppressive and delayed microbiota-dependent immunostimulatory effect

***M. Kverka**[1,2], Z. Jiraskova Zakostelska[1], K. Klimesova[1,3], N. Galanova[1], T. Hudcovic[4], Z. Stehlikova[1], S. Coufal[1], A. Fajstova[1], M. Kostovcik[1], H. Tlaskalova-Hogenova[1];*
[1]Institute of Microbiology of the CAS, Prague, Czech Republic, [2]Institute of Experimental Medicine of the CAS, Prague, Czech Republic, [3]Institute of Molecular Genetics of the CAS, Prague, Czech Republic, [4]Institute of Microbiology of the CAS, Novy Hradek, Czech Republic.

Gut microbiota is critical stimulus for the development of immune system, thus shaping the individual's susceptibility to immune-mediated diseases. Immunomodulatory properties have been reported for some oral antibiotics (ATB). Here, we analyzed if these immunomodulatory properties are microbiota-dependent or -independent. We treated BALB/c mice with daily gavage of either placebo (P), colistin (C), vankomycin (V) or metronidazole (M) for 2 weeks and induced delayed-type hypersensitivity (DTH) during the second week. To analyze the role of microbiota, we performed similar experiment in germ-free conditions, in ex GF mice transferred with gut microbiota or in immunodeficient mice adoptively transferred with leukocytes from P- or M-treated mice. We analyzed effect of ATB on gut microbiota by 16S rRNA gene sequencing, on local or systemic immune response by gut fragment culture or flow cytometry *in vivo* and on TCR stimulation *in vitro.* We found that all ATB changes the gut microbiota composition and decreased DTH. Mice treated with M or V have lower production of pro-inflammatory cytokines in their Peyer's patches, but there were no differences in colons or *in vitro*. Next, we found that M decreases DTH even in absence of microbiota and its effect can be transferred by leukocytes. Interestingly, transfer of microbiota from M-treated mice or by DTH induction delayed by 3 weeks after the last dose of M has opposite effect. We conclude that oral M has microbiota-independent short time immunosuppressive effect but ATB-induced dysbiosis ultimately lead to immune system stimulation. Supported by the Czech Science Foundation (17-09869S)

P.D1.01.09

The effect of probiotic and nasal microbiota on inflammatory response, viral load, and symptom severity in experimental rhinovirus challenge

***M. J. Lehtinen**[1], A. A. Hibberd[2], S. Männikkö[3], N. Yeung[1], T. Kauko[3], S. Forssten[1], L. Lehtoranta[1], S. J. Lahtinen[1], B. Stahl[2], A. Lyra[1], R. B. Turner[4];*
[1]DuPont Nutrition and Health, Kantvik, Finland, [2]DuPont Nutrition and Health, Madison, United States, [3]4Pharma, Turku, Finland, [4]University of Virginia, Charlottesville, United States.

Introduction: Meta-analyses suggest that probiotics could be beneficial on reducing the risk of respiratory infections in humans, however, the role of nasal microbiota, or its modulation by probiotics, in viral respiratory infections has not been established in controlled clinical trial setting. Materials and Methods: We collected nasal swabs and washes, and fecal samples over time, in a randomized double-blind placebo controlled clinical study assessing the effect of prophylactic probiotic *Bifidobacterium animalis* subsp. *lactis* Bl-04 (Bl-04) supplementation on experimental rhinovirus infection in 115 healthy adults (NCT01669603). The nasal and fecal microbiota were characterized by 16S rRNA gene sequencing and the resulting data were compared with nasal inflammatory marker concentrations, viral load, and clinical symptoms during the infection. Results: Probiotic Bl-04 supplementation influenced nasal wash inflammatory response and reduced the viral load during the infection. The sequencing results showed that the nasal microbiota clustered into six types. The clusters predominant of *Staphylococcus, Corynebacterium/Alloiococcus, Moraxella, and Pseudomonadaceae*/Mixed had characteristic inflammatory marker and viral load profiles in nasal washes. The nasal microbiota types of subjects also influenced the severity of clinical cold symptoms during rhinovirus infection. Rhinovirus infection or probiotic intervention did not significantly alter the composition of nasal or fecal microbiota. Conclusions: Our results suggest that probiotic Bl-04 supplementation influences innate inflammatory response and viral load in nasal washes, and that the nasal microbiota type influences the virus load, host innate immune response, and clinical symptoms during rhinovirus infection. This study was funded by DuPont Nutrition and Health.

P.D1.01.10

***B. uniformis CECT 7771* restores the intestinal immune homeostasis in diet-induced obese mice**

***I. López Almela**, M. Romaní Perez, E. Fabersani, I. Campillo, K. Portune, Y. Sanz;*
Microbial Ecology, Nutrition and Health Research Unit, Institute of Agrochemistry and Food Technology, National Research Council (IATA-CSIC), Paterna (Valencia), Spain.

Obesity represents a major health challenge worldwide due to its high prevalence and associated comorbidities. Energy dense diet-induced dysbiosis impairs intestinal immunity which contributes to obesity by promoting systemic inflammation. Probiotic-based therapies are being investigated to combat obesity by promoting a healthy gut microbiota which favors the immune homeostasis and energy balance. Previously we have demonstrated that *Bacteroides uniformis* CECT 7771 has *in vitro* anti-inflammatory properties and restores the metabolic disturbances of diet-induced obese mice. Since the mechanistic understanding of these effects remains unexplored, herein we have investigated the possible immune-mediated effects of this bacterial strain in obesity. Mice were fed with standard or high fat high fructose diet (HFHFD) and orally received the probiotic ($1x10^9$ CFU) or placebo for 14 weeks. The probiotic reduced body weight gain and adiposity and normalized glucose tolerance in HFHFD-fed mice. The obese phenotype was linked to a pro-inflammatory state in blood and white adipose tissue (WAT). Obese mice also showed reduced gene expression of occludin (a gut integrity marker) and enhanced intestinal inflammation; i.e. increase of innate lymphoid cells (ILC)-1 and induced intestinal epithelial lymphocytes (IEL) and reduced ILC3 and natural IEL. The probiotic prevented inflammation in blood, WAT and small intestine and normalized occludin expression in HFHFD-fed mice. Herein, we demonstrated that *B. uniformis* CECT 7771 prevents the impact of the obesogenic diet on intestinal immunity and favours gut integrity. These effects probably contribute to reducing inflammation linked to HFHFD which in turn improves the metabolic phenotype in obesity.

P.D1.01.11

The *Brucella abortus* Cu2+/Zn2+ superoxide dismutase in the Th17 and Treg immune response

***M. C. Moreno-Lafont**[1], H. Velazquez-Soto[1], V. Paredes-Cervantes[2], M. R. Vieyra-Lobato[1], R. Lopez-Santiago[1];*
[1]ENCB-Instituto Politecnico Nacional, Mexico City, Mexico, [2]Hospital General. Centro Medico Nacional La Raza, IMSS, Mexico City, Mexico.

Brucellosis is considered the main zoonosis worldwide, with around half a million cases annually. This infectious disease is caused by intracellular bacteria of the genus *Brucellae*. Currently there is not a vaccine for use in humans and pharmacological treatment for this condition, although it is not expensive is very long. It is for this that the development of vaccines for clinical use is still a very active area in the field of research. The aim of this study consisted in assessing the protective capacity of the SOD Cu2+/ Zn2+ from *Brucella abortus* when administered intragastrically and otherwise evaluate the immunological mechanisms involved in response to this protein. Recombinant SOD was obtained from induction of transformed *E. coli* strain, the protein was purified first by affinity chromatography and subsequently by FPLC. In a murine model, it was shown SOD is able to induce systemic protection against *B. abortus strain 2308* challenge. When exploring the mechanisms that may be involved in the generation of protection, we found that immunized mice produced antibodies capable of recognizing SOD and also these mice increased the percentage of Th17 cells and decreasing the percentage of TGF-β producing regulatory T cells.

P.D1.01.12

Characterisation of CD1a-restricted T cells using CD1a-lipid tetramers

C. V. Nguyen-Robertson[1,2], *S. J. Reddiex*[1,2], *J. M. Cheng*[1,2,3], *A. P. Uldrich*[1,2], *J. Rossjohn*[4,5], *I. Van Rhijn*[6], *S. J. Williams*[3], *B. Moody*[6], *D. I. Godfrey*[1,2], *D. G. Pellicci*[1,2];
[1]*Peter Doherty Institute, The University of Melbourne, Melbourne, Australia,* [2]*ARC Centre in Advanced Molecular Imaging at The University of Melbourne, Parkville, Australia,* [3]*School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Australia,* [4]*Department of Biochemistry and Molecular Biology, Monash University, Clayton, Australia,* [5]*ARC Centre in Advanced Molecular Imaging at Monash University, Clayton, Australia,* [6]*Brigham and Women's Hospital, Division of Rheumatology, Immunology and Allergy and Harvard Medial School, Boston, United States.*

In contrast to conventional T cells that recognise peptide antigens presented by MHC molecules, other T cells recognise lipid antigens presented by MHC-like CD1 family members, CD1a, CD1b, CD1c and CD1d. Recent studies have suggested that CD1a-restricted T cells comprise a unique T cell population in human blood and may also play a unique functional role in skin. Here, we have produced mammalian CD1a tetramers to investigate the phenotype and function of human CD1a-restricted T cells directly *ex vivo*. Interestingly, we have shown that CD1a-restricted T cells that recognise non-self lipid antigens, particularly dideoxymycobactin, a lipid antigen derived from *Mycobacterium tuberculosis*, can also be autoreactive to CD1a.
Additionally, we have defined the T cell receptor (TCR) usage of both self- and foreign-lipid-reactive CD1a-restricted T cells, demonstrating that while they exhibit a diverse TCR repertoire, there is some biased variable gene usage. Experiments with CD1a mutant cell lines revealed that different TCRs can bind across the entire binding cleft of CD1a, which is likely to increase the diversity of lipid antigens that can be recognised by CD1a-restricted T cells. Phenotypic analyses of these cells revealed that they are negative for IL-18R and CD161, markers typically used to define other unconventional, innate-like T cells, such as type I NKT cells and MAIT cells, thereby distinguishing CD1a-restricted T cells from other unconventional T cells. Collectively, these studies represent an important step forward in characterising CD1a-restricted T cells, and further understanding their role in infection and autoimmune responses.

P.D1.01.13

Outbreak of anthrax-like disease in Nigeria from unusual pathogens: preliminary report

S. E. Idachaba, A. G. Rimfa, E. A. Abiayi, G. O. Agada, Y. M. Dashe, ***M. O. Odugbo****;
National Veterinary Research Institute, Vom, Nigeria.*

Introduction: Outbreaks of anthrax disease are making headlines everyday and across the sub regions and continents of the world.
Case Series: An outbreak of anthrax-like disease was reported in April 2016 in three herds of cattle in North Central Nigeria. Clinically, the signs and symptoms observed in the diseased cattle were anthrax-like resulting in four deaths. Microbiologically, the phenotypic characteristics of the pathogens incriminated a co-infection of *Bacillus laterosporus* and *Bacillus pantothenticus* isolated from spleen, blood, and nasal swabs of diseased cattle. *Bacillus anthracis*, the established causative agent of anthrax was not isolated either microscopically, culturally or with use of USA Navy field ELISA test kits for antigen detection; sensitivity of both *Bacillus* species to ciprofloxacin and penicillin were noted. There were reports of skin ulcers on humans that came in contact with infected blood of carcasses although no fatality, perhaps due to intervention using procaine penicillin and local herbs.
Conclusion: Anthrax aetiology is now been represented by quasi-species of *Bacillus*, in addition to traditional *B. anthracis*. Similar reports of anthrax symptoms due to *Bacillus cereus* was recently reported in neighbouring Cameroon and Cote d'Ivoire. Genomic characterization and the virulence of these pathogens in laboratory animals are currently underway. Although vaccination of farm animals with *Bacillus anthracis* spore vaccine strain $34F_2$ has long been the hub of control programmes against anthrax, the recent discoveries of other *Bacillus* species as aetiology of anthrax could undermine the current vaccines in use.
*Presenting author has applied for the "AAI 5th ECI Travel Grant"

P.D1.01.14

alpha-Galactosylceramide administration induces reduction of bacterial load in murine model of brucellosis

O. Rodriguez-Cortes[1], *D. Cabello-Modesto*[2], *A. Hernández-Colín*[3], *R. Soria-Castro*[3], *R. Flores-Mejía*[1], *R. López-Santiago*[3], *M. C. Moreno-Lafont*[3];
[1]*SEPI. Escuela Superior de Medicina. Instituto Politécnico Nacional., México, D.F., Mexico,* [2]*Escuela Superior de Medicina. Instituto Politécnico Nacional., México, D.F., Mexico,* [3]*Deto. Inmunología. Escuela Nacional de Ciencias Biológicas. Instituto Politécnico Nacional, México, D.F., Mexico.*

Brucellosis is a chronic zoonoses whit 500,000 cases annually worldwide. Approximately 50% of the cases have presented with an insidious onset and high rate of chronicity and morbidity. NKTs are non-conventional T lymphocytes which have an invariant TCR to recognize glycolipid, are currently investigated like adjuvants in vaccines and treatment of infectious processes because are an early and strong source of IFN-γ. The aim of this work was determine if systemic activation of NKT lymphocytes induces a decrease in bacterial load in systemic brucellosis model of six weeks old BALB/c female mice infected IP with $3.5x10^6$CFUs *B. abortus* 2308. Groups: a) not treated (NT), b) vehicle (100 µL 1% PBS-tween), c) αGalcer (2 µg αGalcer/100 µL 1%PBS-tween). N=9 in each group. αGalcer and vehicle were IV administrated at -1d, +6d, +13d and +20d of the infection. At +7d, +14d and +21d postinfection a blood sample was taken, animals were sacrificed and was realized extraction of spleen. Were determined splenic index(SI), quantification of NKTs by FACS, CFUs and cytokines in serum by CBA. An increase in SI was observed at 21d in NT group, followed by αGalcer ($P<0.001$); however αGalcer induced a higher number of NKTs/organ at 7d and 21d compared to NT group $9.36x10^5$vs.$6.94x10^5$ and $2.79x10^6$vs.$2.19x10^6$ respectively ($P>0.05$). TNFα and IL-6 serum concentration was higher in αGalCer treated mice at 14d($P<0.001$, $P<0.05$) and a reduction of bacterial load of this group was observed with respect to NT group of $1.1x10^7$vs.$4.73x10^6$ and $2.47x10^6$vs.$5.35x10^5$ at 7d and 21d respectively ($P<0.05$). Grant SIP20170978.

P.D1.01.15

Microbiota promote lesion development during intradermal infection with vaccinia virus

E. V. Shmeleva*, B. J. Ferguson, G. L. Smith;
Department of Pathology, Cambridge, United Kingdom.*

There is a growing interest in the roles of commensal bacteria during viral infections. Vaccinia virus (VACV), which was used to eradicate smallpox, induces skin lesions after intradermal infection, but the pathophysiology of lesion formation is not well studied. While studying lesion formation, we observed a substantial infiltration of neutrophils into VACV-infected ears several days post infection, leading us to hypothesise the presence of secondary bacterial infection. CFU counts revealed a greater than 100-fold increase of bacteria in infected mouse ear tissue in comparison with controls. To investigate the role of skin microbiota in lesion development, mice were treated with a broad-spectrum antibiotic (AB) during infection. AB-treatment halved the size of lesions compared to controls animals, yet the viral titres remained unchanged. FACS analysis of the VACV-infected ear tissues revealed a significant reduction in the recruitment of different subpopulations of myeloid and lymphoid immune cells in AB-treated mice in comparison with controls. Indeed, lesion sizes correlated positively with the number of neutrophils and TCRαβ T cells. Levels of IL-1β, IL-6, TNFα and CCL7 were also reduced by AB treatment compared with controls. In conclusion, skin microbiota increases greatly following intradermal infection with VACV and promotes lesion development and the recruitment of leukocytes to the site of viral infection.

P.D1.01.16

Crohn's disease-associated *Escherichia coli* serotyping through the whole-genome sequencing of cultivated intestinal community

M. Siniagina*, M. Markelova, A. Laikov, E. Boulygina, T. Grigoryeva;
Kazan Federal University, Kazan, Russian Federation.*

Crohn's disease (CD) is chronic inflammatory bowel disease with unclear etiology. Exacerbation of CD is often associated with increase in the number of E.coli.
To characterize the variety of E.coli strains in gut microbiota, stool samples were collected from two patients - with diagnosed Crohn's disease and without any observed symptoms of gastrointestinal disorders (control). Different E.coli morphotypes on Endo medium tested for hemolytic activity as well as mixed colony suspensions from Endo plates were analyzed through whole-genome sequencing on Illumina Miseq platform.
In CD patient 2.76*10^7 CFU and in control 3.8*10^4 CFU were cultured. There were different colony morphotypes in the sample from CD patient represented by O166:H15 (86.7% colonies on plate), O-:H20, O154:H9, O-:H30, O4:H45 serotypes while among control sample morphotypes O6:H1 and O-:H4 serotypes were found. Whole-genome sequencing data of mixed colony suspensions allowed to detect additional E.coli serotypes O8, O68, O159 and O159, O180, O24, O25, O68; H17, H10 and H4, H14 in control and CD sample, respectively.
Thus, abundance of E.coli serotypes in CD sample differs from the control one. Prevalence of O166:H15 serotype, described earlier as an infectious agent caused gastroenteritis outbreak in Osaka (Japan) in 1997, may be associated with CD. This assumption will be checked in future research on an extended cohort of patients using omics technologies.
The reported study was funded by RFBR according to the research project № 17-00-00433.

P.D1.01.17

Effect of Methisoprinol on selected immunological parameters in the course of BRDC treatment in calves

A. K. Siwicki[1], *K. Żarczyńska*[2], *A. Pomianowski*[2], *J. Małaczewska*[1], *R. Wójcik*[1], *P. Schulz*[1];
[1]*Microbiology and Clinical Immunology Department, Veterinary Medicine Faculty, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland,* [2]*Internal Diseases Department and Clinic,Veterinary Medicine Faculty, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland.*

Bovine Respiratory Disease Complex (BRDC) is considered as one of the most important diseases of the respiratory system of calves and young cattle. It is also referred to as enzootic bronchopneumonia of calves (EBC) or shipping fever. BRDC is a polyetiological disease of the respiratory system. This include viruses: bovine herpesvirus-1, bovine respiratory syncytial virus, bovine parainfluenza virus3, bovine coronavirus, bovine adenovirus A-D and bovine viral diarrhea virus 1 and 2. In addition, the role of mycoplasma is emphasized. Bacteria are a secondary factor in the disease.Tests were carried out on 40 calfes qualified on the basis of a clinical trial and confirmation of the presence of at least one virus and one bacterium. Animals were divided into two groups. The experimental group received Methisoprinol three times intramuscularly at two-day intervals (day 1,3 and 5). The second group received a placebo. The level of T cell subpopulations in the plasma were determined. Blood for tests was collected at 1, 9 and 21 day of experiment. Significantly higher values were observed in the methisoprinol group on day 9 and 21 whereas the mean values remained relatively constant in control animals.The study was performed with the approval of the Local Ethics Committee for Experiments on Animal.This study was partially supported by the National Centre for Research and Development - project no: POIG.01.03.01-28-108/12.

P.D1.01.18

Changes in the skin microbiome during allogeneic hematopoietic stem cell transplantation

J. Strobl[1], *N. Bayer*[1], *L. Hammerl*[1], *D. Berry*[2], *V. K. Patra*[3], *G. Stary*[1];
[1]*Medical University of Vienna, Department of Dermatology, Vienna, Austria,* [2]*University of Vienna, Division of Microbial Ecology, Vienna, Austria,* [3]*Medical University of Graz, Department of Dermatology, Graz, Austria.*

The success of allogeneic hematopoietic stem cell transplantation (HSCT) remains limited due to severe side-effects, such as infections and graft-versus-host-disease (GVHD). Recent studies suggest that dysbiosis of intestinal microbes is associated with increased risk of GVHD, while the role of the cutaneous microbiome in this setting remains elusive. We obtained patient material (blood, stool, skin biopsies and -scales) at 5 time points before myeloablative conditioning and up to one year after HSCT (n= 20). The cutaneous and intestinal microbiome is analyzed with 16S ribosomal RNA sequencing. Interactions of bacteria with immune cells are investigated using multiple staining approaches, such as immunofluorescence stainings and fluorescence *in situ* hybridization (FISH). Bacterial numbers/mm² and distance calculations from $CD45^+HLA\text{-}DR^+$ cells are assessed via the StrataQuest Analysis Software (TissueGnostics GmbH).
We successfully established an extraction protocol for microbial DNA from stool and skin scales and can visualize bacteria by 16S rRNA FISH in the epidermis and dermis of skin sections. FISH revealed a decrease in bacterial numbers/mm² skin in the epidermis as well as upper (500µm) and lower (500µm) dermis at day 0 and day 14 after transplantation. Hundred days after HSCT bacterial numbers in skin were comparable to baseline before transplantation. Although often in close contact with $CD45^+HLA\text{-}DR^+$ antigen-presenting cells, no intracellular bacteria were observed. In this new and ongoing project, we aim to build individual risk-profiles for patients based on their skin and gut microbiome and further explore the interaction between the immune system and the residing microbiome in this unique cohort.

P.D1.01.19

Fecal microbiota transplantation is effective to treat intestinal Graft-versus-Host Disease

Y. F. van Lier[1,2,3], *M. Davids*[1], *P. F. de Groot*[1], *E. Nur*[1,3], *S. S. Zeerleder*[1,3,4], *M. Nieuwdorp*[1,5,6], *B. Blom*[1,2], *M. D. Hazenberg*[1,2,3];
[1]*Academic Medical Center (AMC), Amsterdam, Netherlands,* [2]*Amsterdam Infection & Immunity Institute (AI&II), Amsterdam, Netherlands,* [3]*Cancer Center Amsterdam (CCA), Amsterdam, Netherlands,* [4]*Sanquin Research, Amsterdam, Netherlands,* [5]*VU Medical Center (VUMC), Amsterdam, Netherlands,* [6]*University of Gothenburg, Goteborg, Sweden.*

Gut microbiota have a major influence on the intestinal health of allogeneic hematopoietic stem cell transplantation (HSCT) recipients. For example, patients with intestinal dysbiosis after HSCT are more prone to develop Graft-versus-Host Disease (GvHD). Here, we tested if fecal microbiota transplantation (FMT) could restore symbiosis in GvHD patients and thereby would alleviate symptoms. Fifteen allogeneic HSCT recipients with biopsy-proven, steroid-dependent or steroid-refractory intestinal GvHD received a single FMT via nasoduodenal infusion from an unrelated, healthy donor. FMT procedure was well tolerated by all patients and no serious adverse events were observed that could be attributed to FMT. With 6-months follow-up still ongoing, so far 11 out of 15 patients showed improvement of GvHD. Completed follow-up for the first seven patients identified three complete responders (CR). In these patients, defecation frequency and consistency normalized and immunosuppressants were tapered successfully, without relapse of diarrhea. Two partial responders (PR) showed initial improvement of GvHD after FMT but relapsed upon prednisone taper. Analysis of fecal microbiota composition by 16S ribosomal RNA (rRNA) sequencing revealed overall low alpha diversity in patients pre-FMT, with the lowest diversity observed in non-responding participants. Future analysis of collected blood samples and sigmoid biopsies will give further insights into the immune cells involved in the beneficial effect of FMT. This study demonstrates that a single FMT is safe and effective in allogeneic HSCT recipients with steroid-refractory or steroid-dependent GvHD and provides a solid basis to further exploit its effectiveness in larger cohorts.

P.D1.01.20

The enteric pathogen Citrobacter rodentium shapes intestinal bacterial communities to overcome colonization resistance

S. Wirtz, *R. Lakra, M. F. Neurath;*
Medical Department 1, Erlangen, Germany.

The enteric pathogen *Citrobacter rodentium* (CR) is widely used as model for infections with EHEC/EPEC and inflammatory bowel diseases (IBD). While the importance of the T3SS encoded in the locus of enterocyte effacement (LEE) is well established, the functional in vivo role of the endogenous type-VI-secretion-system (T6SS) for *CR* infection remains ill defined. Based on our experimental evidence that the T6SS actively secreted hallmark proteins *in vitro*, we disrupted conserved T6SS genes in a CR reporter-strain. Interestingly, we observed a significantly reduced colonization capacity of these mutants suggesting that CR requires a functional T6SS to infect the intestine efficiency and to exhibit full inflammatory activity. By competition experiments under aerobic and anaerobic conditions using stools/specific bacteria as prey and CR as predator, we found that the T6SS conferred strong interbacterial competition. Notably, this notion was further supported by infection experiments in germ-free mice. Furthermore, a 16S-based next generation sequencing strategy indicated that changes in the microbiome during CR infections largely depend on the presence of a functional T6SS. Collectively, our results suggest that T6SS-dependent effects on bacterial communities allow CR effective colonization of the intestinal ecosystem.

P.D1.01.21

Soluble TNF-like weak inducer of apoptosis (TWEAK) enhances poly(I:C)-induced RIP-mediated necroptosis

M. A. Anany, *J. Kreckel, C. Otto, D. Siegmund, H. Wajant;*
Wuerzburg university hospital, Wuerzburg, Germany.

TNF-like weak inducer of apoptosis (TWEAK) and inhibition of protein synthesis with cycloheximide (CHX) sensitize for poly(I:C)-induced cell death. Notably, while CHX preferentially enhanced poly(I:C)-induced apoptosis, TWEAK enhanced primarily poly(I:C)-induced necroptosis. Both sensitizers of poly(I:C)-induced cell death, however, showed no major effect on proinflammatory poly(I:C) signaling. Analysis of cells lacking TRADD, RIP and FADD or the combination of TRADD and RIP along with caspase-8 KO cells and transfectants expressing FLIPL and FLIPS revealed furthermore similarities and differences in the way how these molecules act in cell death signaling by poly(I:C)/TWEAK and TNF and TRAIL. RIP turned out to be essential for poly(I:C)/TWEAK-induced caspase-8-mediated apoptosis but was dispensable for these responses in TNF and TRAIL signaling. TRADD-RIP double deficiency differentially affected poly(I:C)-triggered gene induction but abrogated gene induction by TNF completely. FADD deficiency abrogated TRAIL- but not TNF- and poly(I:C)-induced necroptosis while TRADD and the two FLIP isoforms elicited protective activity against all three death inducers.Application funding Source
Deutsche Forschungsgemeinschaft (German Research Foundation) - WA1025/30-1 [Wajant] Deutsche Krebshilfe (German Cancer Aid) - 111703 [Wajant] M. A. is a German Egyptian Research Long Term Scholarship (GERLS) holder funded by DAAD. [Anany]

P.D1.01.22

MMP-9-generated COOH-, but not NH_2-terminal fragments of SAA1 retain potentiating activity in neutrophil migration to CXCL8, with loss of direct chemotactic and cytokine inducing capacity

S. Abouelasrar Salama, M. De Buck, J. Vandooren, S. Knoops, N. Pörtner, L. Vanbrabant, G. Opdenakker, P. Proost, J. Van Damme, S. Struyf, ***M. Gouwy****;*
Rega Institute, Leuven, Belgium.

Serum amyloid A1 (SAA1) is an acute phase protein, induced to extremely high levels upon inflammation and infection. Human SAA and its NH_2-terminal part are studied extensively in the context of amyloidosis. In contrast, little is known about COOH-terminal fragments of SAA. Intact SAA1 chemoattracts leukocytes via the G protein-coupled receptor formyl peptide receptor like 1/formyl peptide receptor 2 (FPRL1/FPR2). In addition to direct leukocyte activation, SAA1 induces chemokine production by signaling through TLR2. We recently discovered that these induced chemokines synergize with intact SAA1 to chemoattract leukocytes. Gelatinase B or matrix metalloproteinase-9 (MMP-9) is induced by SAA1 during infection and inflammation. We here demonstrate that processing of SAA1 by MMP-9 yielded predominantly three COOH-terminal fragments: SAA1(52-104), SAA1(57-104) and SAA1(58-104).

To investigate the effect of proteolytic processing on the biological activity of SAA1, we chemically synthesized the COOH-terminal SAA fragments SAA1(52-104) and SAA1(58-104) and the complementary NH_2-terminal peptide SAA1(1-51). In contrast to intact SAA1, the synthesized SAA1 peptides did not induce interleukin-8 (IL-8)/CXCL8 in monocytes or fibroblasts. Moreover, these fragments possessed no direct chemotactic activity for neutrophils, as observed for intact SAA1. However, comparable to intact SAA1, SAA1(58-104) synergized with CXCL8 in neutrophil activation and migration, whereas SAA1(1-51) lacked this synergistic activity. This synergistic interaction between the COOH-terminal SAA1 fragment and CXCL8 in neutrophil chemotaxis was mediated by FPR2. Hence, proteolytic cleavage of SAA1 by MMP-9 fine-tunes the inflammatory capacity of this acute phase protein in that only the synergistic interactions with chemokines remain to prolong the duration of inflammation.

P.D1.02 Microbiome, metabolites and the immune system - Part 2

P.D1.02.01

Excretion-secretion products from *Taenia crassiceps* cysticerci induce regulatory T cells

L. Adalid-Peralta[1], *D. López Recinos*[1], *V. Morales Ruiz*[1], *M. G. Castañeda Torrico*[1], *A. Guevara Salinas*[1], *S. Gómez Fuentes*[1], *C. Parada Colín*[2], *C. Espitia Pinzón*[2], *M. Hernández González*[2], *I. Mora Herrera*[2], *G. Fragoso*[2], *E. Sciutto*[1];
[1]*Instituto Nacional de Neurología y Neurocirugía, Mexico City, Mexico,* [2]*Instituto de Investigaciones Biomédicas, UNAM, Mexico City, Mexico.*

Introduction: Neurocysticercosis is caused by the establishment of *Taenia solium* larvae in the central nervous system. The *T. crassiceps* cysticercosis murine model has allowed us to study *T. solium* infections. While the parasite is known to induce regulatory T cells (Tregs), the components involved in Treg induction are unknown. This work is aimed to identify and characterize Treg-inducing excretion-secretion products (ESP) by cellular, proteomic, and bioinformatic analysis.
Materials and Methods: ESP were obtained from supernatants of *T. crassiceps* cysticercus cultures in DMEM at 120 days post-infection. ESP were dialyzed, lyophilized, and quantified. For Treg induction, 0, 250, 500, 700, or 1000 µg of protein of each extract per mouse were inoculated intraperitonially. Five days after inoculation, mice were sacrificed, peritoneal cells were obtained, and Treg percentage was evaluated by flow cytometry. Peptide fingerprint was determined by 2-dimension gels. The spots were cut and digested for mass spectrometry analysis, and the resulting peptides were characterized by bioinformatics.
Results: From the six different ESP analyzed, four were identified as Treg-inducing and two as non-Treg-inducing. The isoelectric point and molecular weight of one Treg-inducing and one non-Treg-inducing ESP were compared, and 34 differential spots were identified for the Treg-inducing ESP-6. Spots were sequenced, and 21 candidate proteins were identified and classified by function in metabolic and immunological processes.
Conclusions: ESP from *T. crassiceps* include Treg-inducing proteins, which could participate in the establishment and permanence of cysticerci in an immunocompetent host. These ESP could provide new therapeutic tools against chronic inflammation in human diseases.

P.D1.02.02

Immunemodulatory effects of malaria co-infection with *Mycobacterium ulcerans* disease in Ghana

D. Antwi-Berko[1,2], *N. Nausch*[3], *W. Owusu*[1], *E. Owusu-Dabo*[1], *L. Batsa-Debrah*[1], *A. Y. Debrah*[1,4], *M. Jacobsen*[3], *R. O. Phillips*[1,5];
[1]*Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana,* [2]*Department of Basic and Applied Biology, University of Energy and Natural Resources, Sunyani, Ghana,* [3]*Pediatric Pneumology and Infectious Diseases Group, Department of General Pediatrics, Neonatology, and Pediatric Cardiology, University Children's Hospital, Heinrich-Heine University, Duesseldorf, Germany,* [4]*Faculty of Allied Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,* [5]*Department of Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.*

Introduction: Buruli ulcer disease (BUD) caused by *Mycobacterium ulcerans* (*Mu*) is endemic in tropical regions; hence there is a concomitant risk of co-infection with other infections including *Plasmodium falciparum* (malaria). However, immunomodulatory effect of malaria co-infection among BUD patients has not been elucidated. This study sought to determine the immune-modulation by malaria and its impact on host immunity and clinical presentation of BUD in children.
Methods: This observational study recruited 42 children with BUD (1.5-17 years) and 29 healthy contacts (2-15 years) from the Agogo Presbyterian Hospital and screened serologically for *P. falciparum*. Heparinized whole blood samples of participants were stained and CD4+ T cells analyzed for expression of CXCR3, CD161, and CRTH2 receptors. After overnight stimulation with *Mu* antigen, CD4+ T cells were analyzed for TNFα, IFNγ, CD40L, IL-5, GM-CSF, IL-4, IL-9, IL-22, IL-17A, IL-10, IL-2 and IP-10 levels by flow cytometry. Proportions and profile of chemokine receptors/cytokine producing CD4+ T cells was compared and correlated with disease progression and severity.
Results: 50% of BUD patients and 58% of healthy contacts were sero-positive for malaria. Malaria positive BUD patients showed significantly reduced CRTH2+CXCR3+ CD4+ T cells, elevated proportions of CD40L+ and IFNγ-TNFα+ whilst there was non-significant increased TNFα+ and CD40L-TNFα+ producing CD4+ T cells compared to BUD/malaria sero-negatives. IFNγ-TNFα+ producing CD4+ T cells correlated with the widest diameter of lesions in BUD/malaria sero-positive patients.
Conclusion: These findings suggest that the increase in IFNγ-TNFα+ and CD40L+ producing T cell subset may reflect a strong inflammatory response in BUD patients.

P.D1.02.03

An *in vivo* [^{11}C] PBR28 positron emission tomography (PET) study of microglia and astrocytes cells activation in pneumococcal meningitis.

T. Barichello[1], *V. V. Giridharan*[1], *G. Scaini*[1], *J. S. Generoso*[2], *L. R. Simoes*[2], *A. Collodel*[2], *D. Dominguini*[2], *S. Sudhakar*[1], *F. Petronilho*[3], *F. Dal-Pizzol*[2];
[1]*The University of Texas Health Science Center at Houston, Houston, United States,* [2]*Universidade do Extremo Sul Catarinense - UNESC, Criciuma, Brazil,* [3]*Universidade do Sul Catarinense - UNISUL, Tubarao, Brazil.*

Translocator protein (TSPO) is localized in the outer membrane of the mitochondria and it is expressed in activated microglia and astrocytes cells. TSPO interacts with voltage-dependent-anion channels (VDACs) and adenine-nucleotide translocators (ANTs) thereby increasing the permeability of the mitochondrial membrane facilitating cardiolipin oxidation and cytochrome-c liberation. *Aim:* to quantify neuroinflammation in terms of fixation and distribution of [^{11}C]-PBR28 and to study its relationship with cognitive impairment after pneumococcal meningitis. At 24 h and 10 days after pneumococcal meningitis induction, Wistar rats were subjected to PET with [^{11}C]-PBR28, a radiotracer with high binding specificity for the TSPO. The expression of cytokines, TSPO, VDAC, ANT, cytochrome-c, microglia and astrocytes markers were evaluated in the rat brain. The 10 days group was subjected to behavior task and was evaluated the aforementioned biomarkers. *Results: 24 h group:* IL-1α, IL-1β, IL-6, IL-18, IFN-γ, and TNF-α levels (Bioplex) and the microglial marker ionized calcium-binding adapter molecule-1 (Iba-1), cluster of differentiation molecule-11b (CD-11b), astrocyte marker glial fibrillary acidic protein (GFAP), cytochrome-c, TSPO expression were higher compared with control group (Western-blot/HIC). In the PET imaging all brain structures presented a higher uptake of [^{11}C]-PBR28. *10 days group:* The meningitis group presented impairment of habituation, aversive, and long-term recognition memories. In amygdala, hippocampus, and thalamus uptake of [^{11}C]-PBR28 were higher in the meningitis group. TNF-α levels, cytochrome-c, Iba-1, CD-11b, TSPO, and GFAP expression were higher in the brain 10 days after meningitis induction. Pneumococcal meningitis survivor's rats presented cognitive impairment that was associated with microglia and astrocyte cells activation.

P.D1.02.04

Gut microbiota is associated with the responsiveness of *in vitro* generated human dendritic cells to stimuli

J. Djokic[1], *D. Mihajlovic*[2], *M. Tolinacki*[1], *S. Bojic*[3], *B. Pavlovic*[4], *D. Vucevic*[2], *N. Golic*[1], *M. Colic*[5], *S. Tomic*[5];
[1]*Laboratory for molecular microbiology, Institute for Molecular Genetics and Genetical Engineering, University in Belgrade, Belgrade, Serbia,* [2]*Medical Faculty of the Military Medical Academy, University of Defence, Belgrade, Serbia,* [3]*HITTest, d.o.o., Belgrade, Serbia,* [4]*Pharmaceutical Faculty, University in Belgrade, Belgrade, Serbia,* [5]*Institute for the Application of Nuclear Energy, Belgrade, Serbia.*

The properties of *in vitro* generated dendritic cells (DC) vary greatly between different donors, leading to variable success upon their application in immunotherapies. Although gut microbiome was shown to influence the efficacy of various immunotherapeutic approaches, similar studies for *in vitro* generated DC are missing. Here we included 15 heathy donors to analyse association of gut microbiota by 16S rRNA sequencing with the responsiveness of their monocyte-derived DC to lipopolysaccharide (LPS) and IFN-γ stimulation. Although all DC down-regulated completely CD14 expression after the differentiation, their responsiveness to LPS/IFN-γ stimulation varied greatly, considering the expression of maturation markers and cytokines production. The expression of ILT-3 by DC negatively correlated with CD83 and CD86 expression, as well as IL-12p70 production. Additionally, the increase in pro-tumorigenic IL-22 production by DC, negatively correlated with the production of proinflammatory cytokines (IL-12p70, TNF-α, IL-6) after LPS/IFN-γ treatment. The frequency of *Sutterella* in feces correlated positively with the capacity of DC to produce IL-12p70, and negatively with expression of ILT-3.
Additionally, the up-regulation of ILT-3 by DC correlated negatively with the frequency of *Lachnospiraceae* in the gut microbita. In contrast, the amount of short-chain fatty acids (SCFA) and the frequency of SCFA producers in gut microbiota, correlated negatively with the capacity of DC to produce proinflammatory cytokines production after stimulation. These results showed that gut microbiota significantly affect the capacity of *in vitro* generated DC to respond to stimuli, suggesting that the manipulation of gut microbiota in patients might improve the immunotherapeutic protocols based on DC vaccines.

P.D1.02.05

Effects of human gut mycobiome *in vitro*

N. Galanova[1], *L. Bajer*[1,2], *M. Kostovcik*[3], *M. Kolarik*[3], *M. Kverka*[1], *K. Klimesova*[1];
[1]*Laboratory of cellular and molecular Immunology, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic,* [2]*Department of Gastroenterology and Hepatology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic,* [3]*Laboratory of fungal genetics and metabolism, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic.*

Although the connection between microscopic fungi and inflammatory bowel disease (IBD) has been discussed ever since the discovery of anti-*Saccharomyces cerevisiae* antibodies, the mechanisms of action on gut inflammation have been studied only marginally. We therefore focused on the proinflammatory potential of the gut fungi. We cultivated fresh fecal samples of IBD patients and healthy controls and isolated morphologically different fungal colonies. These we dereplicated using primer Eric1R and identified by Sanger sequencing. Finally, we processed them into lysates, which we used for stimulation of various cells, such as immune and epithelial cell lines and primary cell cultures. We analyzed their effects using flow cytometry and ELISA. The preliminary data on RAW 264.7 cells showed increased proliferation with reducing concentration of the stimuli. The only exception was stimulation by filamentous fungi *Mucor plumbeus,* which also increased MHCII and IL-6 response. The yeast *Candida parapsilosis* induced the strongest immune response associated with increased expression of costimulatory molecules such as CD80, CD86 and CD40. Proinflammatory response was further validated by increased TNF-α and IL-6 production. Interestingly, the highest stimulation was exhibited by *C. parapsilosis* isolated from healthy control, which in several parameters, e.g. CD80, iNOS, NO_2^- and IL-6, exceeded the stimulation by positive controls. We have shown the proinflammatory effect of *Candida parapsilosis* and *Mucor plumbeus* on the RAW cells. Interestingly, different isolates of the same species induced dissimilar response, emphasizing the complexity of involved mechanisms. The research was funded by GAUK (1366217), GACR (17-066324 and 17-09869S), AZV (15-27580A and 15-28064A).

P.D1.02.06

Gut Flora, MAIT cells and Multiple Sclerosis

F. Gargano[1], *B. Serafini*[2], *L. Rizzetto*[3], *M. Buscarinu*[4], *G. Guerrera*[1], *E. Piras*[1], *V. Annibali*[5], *M. De Bardi*[1], *S. Ruggieri*[6], *C. Gasperini*[6], *G. Ristori*[5], *G. Borsellino*[1], *L. Pavarini*[7], *D. Cavalieri*[7], *M. Salvetti*[5], *C. De Filippo*[8], *L. Battistini*[1], *D. Angelini*[1];
[1]*Neuroimmunology Unit, Fondazione Santa Lucia, Rome, Italy,* [2]*Istituto Superiore di Sanità, Department of Cell Biology and Neuroscience, Rome, Italy,* [3]*Nutrition and Nutrigenomics research group Food Quality Nutrition & Health Department Research and Innovation Centre - Fondazione Edmund Mach, S. Michele all' Adige, Trento, Italy,* [4]*4Neurology and Centre for experimental Neurological therapies (CENTERS), S. Andrea Hospital, Sapienza University, Rome, Italy,* [5]*Neurology and Centre for experimental Neurological therapies (CENTERS), S. Andrea Hospital, Sapienza University, Rome, Italy,* [6]*Department of Neuroscience "Lancisi", S. Camillo Hospital, Rome, Italy,* [7]*University of Florence, Department of Biology, Florence, Italy. 7 CNR Institute IBIMET, Florence, Italy,* [8]*CNR Institute IBIMET, Pisa, Italy.*

The composition of the intestinal microbiota plays a critical role for the shaping of the immune system. Recent studies suggest that the microbiota may have a role in immune-mediated central nervous system diseases such as multiple sclerosis (MS).
We find that a distinct population of lymphocytes, named MAIT (mucosal-associated invariant T cells), is expanded in individuals with MS. We collected faecal samples from 27 MS patients and 18 healthy subjects (HS) and we studied the composition of the cultivable gut mycobiota. We find a tendency towards higher fungal abundance and richness in the MS group. Analyzing MS-twin and HS-twin individuals, we observed that MS twins show a much higher rate of *S. cerevisiae (SC)*, and a decrease of Candida Albicans (CA).
We then studied the response of MAIT cells to SC and CA strains isolated from faecal samples. Multicolour flow cytometry was used to study MAIT cells' responses. We find that MAIT cells from MS patients are significantly more activated and have higher proliferative rates than those from healthy donors. We find that MAIT cell activation and proliferation are mediated by IL-12 and IL23 produced by monocytes.
Finally, immunohistochemistry of MS post mortem brains show that MAIT cells can cross the blood-brain barrier and produce pro-inflammatory cytokines in the brain. These results are in agreement with the hypothesis that dysbiosis of the gut microbiota may determine a dysfunction of mucosal responses and may favour the development of systemic inflammatory and autoimmune diseases.

P.D1.02.07

Antileishmanial and immunodulatory effect of Babassu-loaded PLGA micro and 1 nanoparticles: An useful drug target to Leishmania amazonensis infection

R. N. M. Guerra[1], *M. C. Silva*[1], *F. R. Nascimento*[1], *R. Nicolette*[2], *J. M. Brito*[1], *A. M. Vale*[1], *A. P. Santos*[1], *P. V. Soeiro*[1];
[1]*Federal University of Maranhão, São Luís, Brazil,* [2]*FIOCRUZ CEARA, Fortaleza, Brazil.*

Introduction: It was evaluated the immunological and the anti-*Leishmania amazonensis* activity of babassu-loaded *poly [lactic-co-glycolic acid) [*PLGA] micro/nanoparticles. Material and Methods: The Anti- *Leishmania* activity was evaluated against promastigotes or amastigotes forms, in Balb/c macrophages. Results: The size of the micro/nanoparticles ranged from 3 to 6.4 µm, with a zeta potential of −25 mV and encapsulation efficiency of 48%. The anti-*Leishmania* activity of MMP (IC_{50}) was 10-fold higher than that free extract (Meso). MMP exhibited overall bioavailability and was very effective in eliminating intracellular parasites. MMP also reduced *ex vivo* parasite infectivity probably by the increased the production of nitric oxide, hydrogen peroxide and TNF-alfa indicating the activation of M1 macrophages. The over expression of TNF-alfa did not impair cell viability, suggesting anti-apoptotic effects of MMP. Conclusions: These findings indicate that babassu-loaded micro/nanoparticles could be useful for drug targeting because of their immunomodulatory effects on macrophage polarization and the increased efficacy as anti-*Leishmania* product.

P.D1.02.08

Dietary modification reduces multiple sclerosis-like disease in adult marmoset monkeys

Y. Kap[1], *H. Harmsen*[2], *B. Eggen*[2], *J. Bauer*[3], *N. van Driel*[1], *C. Bus-Spoor*[2], *M. Dubbelaar*[2], *C. Grit*[2], *S. Kooistra*[2], *J. Laman*[2], *B. 't Hart*[1];
[1]*Biomedical Primate Research Centre, Rijswijk, Netherlands,* [2]*University Medical Center Groningen, Groningen, Netherlands,* [3]*Brain Research Institute, Vienna, Austria.*

After the introduction of a new dietary supplement (yoghurt-based and vitamin-enriched) in our marmoset colony, the frequency of marmosets in which clinically evident experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (MS), could be induced had decreased from 100 to ~60%. This finding prompted the here reported controlled study in marmoset twins where the effects of the new and classic dietary supplement on factors contributing to EAE susceptibility were compared. One sibling of eight adult twin pairs raised on the new diet were fed the classic diet starting eight weeks before EAE induction with rhMOG/IFA; the other sibling was maintained on the new diet. In the monkeys reverted to the classic diet a 100% EAE incidence was observed. In monkeys fed the new diet the EAE incidence was 75%, spinal cord demyelination was significantly lower, RNA-sequencing analysis of CNS tissue provided evidence for reduced apoptosis and enhanced myelination. In addition, a reduced autoimmune response to the immunized protein was observed in new diet animals. Next, we analyzed whether diet-related changes in microbiota were detectable and associated with disease progression. Despite the dietary modification, twin siblings displayed an essentially unaltered microbiota composition before EAE induction. However, three weeks after immunization, divergence of the fecal microbiota composition between both groups was first detectable, which was even more clear seven weeks after immunization. In conclusion, we report a marked effect of dietary modification on the CNS and gut microbiota and thereby affecting the susceptibility of adult, outbred, conventionally-housed marmosets to MS-like disease.

P.D1.02.09

The combined effect of probiotic and chondroitin sulfate on the levels of cytokines and matrix metalloproteinases in the serum of rats with monoiodoacetate-induced osteoarthritis

O. Korotkyi, *A. Vovk, T. Halenova, K. Dvorshchenko, O. Savchuk, T. Falalyeyeva, L. Ostapchenko;*
ESC "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv, Kyiv, Ukraine.

Cytokines (CK) and matrix metalloproteinases (MMP) play a major role in the pathogenesis of osteoarthritis (OA). The recent studies showed the effect of probiotics (PBs) and chondroitin sulfate (CS) on inflammatory processes during OA, but available results are limited and conflicted. We used a single injection of monoiodoacetate through the infrapatellar ligament of Wistar male rats to start OA model. Therapeutic groups got an intramuscular injection of CS ("Drastop", World Medicine, UK) daily from 2nd to 29th days of experiment and intragastric feeding of PB ("Symbiter" O.D. Prolisok, Ukraine) from 8th to 21st days of the experiment. Sampling was provided on the 30th day. The levels of CKs (interleukins (ILs) IL-1β, IL-4, IL-10, IL-12β, tumor necrosis factor (TNF)-α, interferon (IFN)-γ) and MMPs (MMP-1, MMP-2, MMP-3, MMP-8) were measured in serum by enzyme-linked immunosorbent assay (Biotrak ELISA System, Healthcare, USA). A histologic analysis of knee cartilage tissue made followed according to international recommendation (hematoxilin&eosin, x200). OA caused a significant increase the level of all CKs and MMPs (except IL-4, it was in norm) comparing to control group. Separated administration of CS and PB has decreased all parameters, but they did not reach the control group. PB effect was less significant than CS. The combined administration of PB and CS significant decreased the levels of CKs and MMPs and it was approaching to control values. This data has correlated with histological findings. Thus, the synergetic action of PB and CS is promising and further investigations are waited with interest.

P.D1.02.10

Different gut microbial communities in murine asthma model with obesity

G. Gu, W. Chang, K. Kim, S. Lee, S. Kim, J. Lim, ***J. Lee****;*
Division of Allergy and Chronic Resipiratory Diseases, Center for Biomedical Sciences, Korea National institute of Health, Korea Centers for Disease Control and Prevention, Osong, Chungcheongbuk-do, Korea, Republic of.

Introduction: Obesity is considered as a risk factor for asthma. However, the mechanisms linking obesity and asthma are poorly understood. Recently, it is reported that obesity-related alterations in gut microbiota contributes to obesity-related asthma. To address the relationship between alteration of gut microbiota and pathogenesis of asthma, we analyzed the gut microbiota in murine model.
Material and Methods: Male C57BL/6 mice were fed with a high-fat diet or Low-fat diet to induce obese, and then were exposed to house dust mite extract to induce the lung inflammation. Disease severity was assessed by measuring airway hypersensitivity, infiltration of immune cell in lung, serum immunoglobulin, and cytokine production. In addition, microbial community analysis were performed on cecum via 16S rRNA pyrosequencing.
Results: First, we found that obese-asthma group exhibited significantly increased airway responsiveness and infiltration of immune cells. Total IgE and cytokine production were slightly increased in obese-asthma group compared to chow group, but they have no significance. Second, the composition of gut microbiota was showed to be clustered according to groups. Diversity and richness of gut microbiota was decreased in obese-asthma group than in chow group. Lastly, *Roseburia, Lachnospiraceae, Oscillibacter, Hydrogenoanaerobacterium, Ruminococcacea,* of Firmicutes were decreased in obese and/or asthma group, whereas *Enterococcus* of Firmicutes were increased in obese and/or asthma group.
Conclusion: Our finding suggest that alterations in gut microbiota might be associated with severity of obese-asthma. Further studies on the major effector bacteria in obese-asthma pathogenesis will be necessary to clarify the association of gut microbiome with the obese-asthma.

P.D1.02.11

Streptococcal pyrogenic exotoxin A induces regulatory T Cells via TNFa-TNFR2 signaling

J. Ma[1]*, C. Lu*[1]*, M. Lin*[2,3]*, C. Chiang-Ni*[1,2]*, M. Kuo*[1,2]*;*
[1]*Graduate Institute of Biomedical Sciences, Chang Gung University, Taoyuan City, Taiwan,* [2]*Department of Microbiology and Immunology, Chang Gung University, Taoyuan City, Taiwan,* [3]*Institute of Molecular Medicine, National Taiwan University, Taipei city, Taiwan.*

Introduction: Superantigens non-specifically cross-link T cell receptor and MHC class II to prompt T cell proliferation and activate antigen presenting cells. Several studies indicated that superantigens can induce regulatory T (Treg) cells proliferation. Tregs are characterized as $CD4^+CD25^{hi}Foxp3^+$ cells, whereas Foxp3 expression is important for Tregs function. Although only 1~2% of $CD4^+$ T cells are Tregs, they play critical roles on regulating immune responses. Cytokines such as IL-2, IL-10, TGF-β and TNF-α play important roles to support Tregs expansion and suppressive functions. In this study, we tested whether bacterial superantigen, streptococcal pyrogenic exotoxin (SPE) A, can induce functional Tregs and understanding possible mechanisms. Material and methods: Peripheral blood mononuclear cells were co-cultured with SPEA and Tregs population was analyzed by flow cytometry. The suppressive function of SPEA-induced Tregs was determined by culturing with anti-CD2/CD3/CD28 Dynabeads activated $CD4^+$ T cells. In order to investigate the potential modulatory pathway, neutralizing antibodies were used to test whether TGF-β, IL-10 or TNF-α contributes to the induction of Tregs. Results: SPEA increased $CD25^+Foxp3^+$ cells with the presence of antigen presenting cells. These SPEA-induced Tregs remain their suppressive function and down-regulates IL-2, which is an important cytokine for T cell activation. TGF-β and IL-10 were not involved in $CD25^+Foxp3^+$ cells induction, while neutralizing TNF-α or blocking TNF-α receptor 2 (TNFR2) reduced the population of $CD25^+Foxp3^+$ cells. Conclusions: This study implicated that TNFR2 signaling was involved in SPEA stimulating functional Tregs. This result suggests a new option for drug design against autoimmune diseases or graft transplantation.

P.D1.02.12

Commensal bacteria drive systemic dendritic cell maturation via induction of type-I interferons

S. Muth[1,2]*, L. Rogell*[3,2]*, S. Lienenklaus*[4,5]*, U. Kalinke*[6]*, H. Schild*[1,2]*, A. Diefenbach*[7,3,2]*, H. C. Probst*[1,2]*;*
[1]*University Medical Centre Mainz, Institute for Immunology, Mainz, Germany,* [2]*University Medical Centre Mainz, Research Centre for Immunotherapy (FZI), Mainz, Germany,* [3]*University Medical Centre Mainz, Institute of Medical Microbiology and Hygiene, Mainz, Germany,* [4]*Helmholtz Centre for Infection Research, Molecular Immunology, Braunschweig, Germany,* [5]*Hannover Medical School, Institute for Laboratory Animal Science, Hannover, Germany,* [6]*TWINCORE, Institute for Experimental Infection Research, Hannover, Germany,* [7]*Charité - Universitätsmedizin Berlin, Department of Microbiology, Berlin, Germany.*

Dendritic cell activation is essential for the priming of adaptive immune responses. To ensure immune homeostasis T cell activation by dendritic cells in the immuological steady state must be prevented. We have recently shown that direct interaction of FoxP3+ regulatory T cells with dendritic cells is essential to keep dendritic cells in a resting state. In the absence of regulatory T cell-mediated suppression dendritic cells lose their immature phenotype and activate naive T cells rather than inducing peripheral T cell tolerance, which finally leads to the development of autoimmunity. Here, we sought to identify the activating signals that act on DC in the steady state and drive their maturation when not counterbalanced by regulatory T cells. Using a transgenic mouse model that allows inducible expression of CTL epitopes in dendritic cells without influencing their activation state we identify the commensal flora as a driver of DC activation in the steady state. We show that activation is independent of direct pathogen recognition receptor signaling on the DC but critically depends on tonic levels of type I interferons, which are induced in response to the commensal flora. Our data highlight how the activation threshold for adaptive immune responses is set by the DCs continuous integration of activating and inhibitory signals and identify commensal induced type I interferons and regulatory T cells as opposing forces in this balance.

P.D1.02.13

Prophylactic treatment with simvastatin modulates the immune response and increases animal survival following lethal sepsis infection

J. F. Braga-Filho[1]*, A. G. Abreu*[2]*, C. P. Rios*[1]*, D. Luz*[1]*, D. Cysne*[1]*, J. R. Nascimento*[1]*, L. A. Silva*[1]*, T. S. Fortes*[1]*, R. N. Guerra*[1]*, M. C. Maciel*[1]*, C. Serezani*[3]*,* ***F. R. F. Nascimento***[1]*;*
[1]*UFMA, Sao Luis, Brazil,* [2]*UNICEUMA, Sao Luis, Brazil,* [3]*Vanderbilt University School of Medicine, Nashville, United States.*

Statins may have anti-inflammatory action, promoting immunomodulation and survival in patients with sepsis. The aim here was to analyze the effects of pretreatment with simvastatin in lethal sepsis induced by cecal ligation and puncture (CLP) in mice.
Swiss mice received prophylactic treatment with simvastatin in a single daily dose for 30 days. Then the CLP was performed and the survival was monitored. A half of mice was euthanized after 12h to analyze hematological parameters; cytokines production; cellular influx to peritoneum and bronchoalveolar lavage (BAL); colony-forming units (CFUs); lymphoid organs cellularity; immunophenotyping of T cells and antigen presenting cells and nitric oxide (NO) and hydrogen peroxide (H_2O_2) production.
Simvastatin induced an increase in survival, a decrease in the CFU count on the peritoneum, and a decrease in cells in BAL, in particular lymphocytes. In the blood, there were no differences in the total leukocyte count, but there was a relative lymphocytosis and thrombocytosis in the simvastatin group. The simvastatin induced a greater activation and proliferation of CD4+ T cells in the mesenteric lymph node, as well as an increase in IL-6 and MCP-1 production. The simvastatin induced a decrease in chemotaxis to the peritoneum, but there was an increase in NO and H_2O_2 secretion. Thus, these data suggest that treatment with simvastatin has an impact on the survival of animals, as well as immunomodulatory effects in sepsis induced by CLP in mice.Supported by: FAPEMA, CNPq and CAPES.

P.D1.02.14

Comparative evaluation of microbial translocation products (LPS, sCD14, IgM Endocab) in HIV-1 infected Indian Individuals

N. Negi*, R. Singh, A. Sharma, B. K. Das, M. Vajpayee;*
All India Institute of Medical Sciences, New Delhi, India.

Introduction: Microbial translocation of lipopolysaccharides (LPS), soluble CD14 and Endocab levels have been associated with disease progression during HIV-1 infection. In this longitudinal study, plasma levels of different microbially translocated products (LPS, sCD14, Endocab) was investigated in HIV-1 infected Indian Individuals further stratified as Rapid (R), Viremic slow (VS), Slow progressors (S) and healthy controls.
Materials and Methods: Ten healthy and twenty HIV-1 infected individuals were enrolled. Plasma levels of LPS, sCD14, Endocab was examined using commercially available Limulus Amebocyte assay and enzyme-linked immunosorbent assay (ELISA) enzyme linked immunosorbant assay.
Results: Elevated levels of soluble CD14, IgM EndoCab and LPS were observed in HIV-1 infected individuals compared to healthy controls. Rapid progressors had higher levels of sCD14, IgM EndoCab, LPS (median% 1553, 3596, 202.2) compared to viremic slow, slow progressors and healthy controls both at baseline and follow up visits. At baseline, LPS correlated positively with IgM Endocab and negatively with sCD14 levels while at follow significant positive correlation was observed with IgM Endocab and sCD14 (IgM EndoCab r= 0.490, p=0.05; sCD14 r= 0.051, p= 0.830). Plasma levels of soluble CD14 correlated positively with viral load in rapid, viremic slow and slow progressors while CD+ T cell count correlated positively with soluble CD14 and IgM EndoCab levels in viremic slow and slow progressors.
Conclusion: Our findings indicate that elevated levels of soluble CD14, IgM EndoCab and LPS in HIV-1 infected individuals are strong predictors of disease progression and could be considered as candidate biomarkers for disease monitoring.

P.D1.02.15
Differences in immune responses among unvaccinated adults

***R. M. Noah**;*
Universiti Kuala Lumpur, Kajang, Malaysia.

Outbreaks in certain countries of classified vaccine-preventable infections stormed the headlines for the past 5 years. Countries most affected are those with the influx of immigrants and illegal foreign workers in which majority have not received any childhood vaccinations. The Malaysian Health Ministry issued statement of great concern in the increase of 15% death caused by tuberculosis closely linked to the increase in the number of foreign workers in the country. The investigation was conducted to evaluate the immune function of unvaccinated foreign workers in view of the question whether the immune status in the unvaccinated adults contribute to the transmission of the microbial infections to the vaccinated local populations. Twenty foreign workers and 20 local residents volunteered in the study. Oxidative bursts of lymphocytes were assessed by chemiluminescence assay while a colorimetric assay was used to determine the cellular proliferation of lymphocytes. To analyse the innate immunity status, levels of specific antibodies were measured. There was a significant increment in the oxidative stress of lymphocytes isolated in 60.0% of the unvaccinated individuals. In the proliferative assessment of lymphocytes, 50.0% of the samples recorded absence or lack of proliferation. The humoral immune response on the other hand displayed a significant difference between the unvaccinated and the vaccinated adults suggestive of the effects in individuals not receiving any form of vaccinations. Variations in the immunity of the unvaccinated adults could be responsible for the emergence of microbial infections. These individuals are also at risk of developing complications in non-communicable diseases.

P.D1.02.16
Plasmacytoid dendritic cells regulate inflammation during dysbiosis

***S. Pöysti**, S. Silojärvi, A. Hänninen, R. Toivonen;*
University of Turku, Turku, Finland.

Dendritic cells (DC) are first in line to sense invading microbes and to deliver the signal to other immune cells. Plasmacytoid dendritic cells (pDC) are one subset of DCs and are mainly known for their ability to produce high amounts of type I interferons (IFN). The role of pDCs in bacterial infections is still poorly understood. Our recent studies show high pDCs activation after *Citrobacter rodentium* infection in colon draining mesenteric lymph nodes (coMLN). Here we show an essential role of pDCs in regulating immune response to dysbiosis using a specific pDC-depleted mouse model and *Citrobacter*-induced dysbiosis. We found that dysbiosis had a more severe effect on pDC-depleted mice when compared to wild type mice. Deficiency of pDC during dysbiosis caused 20 % weight loss when no change in wild type mice were seen. Colon epithelium was damaged, epithelial stress genes were upregulated and overall colon length was shorter in pDC depleted mice. T cell analysis showed that the lack of pDCs during dysbiosis blocks the induction of Foxp3+ regulatory T cells in coMLN and increases IFNγ production by both CD4 (T helper) and CD8 (T cytotoxic) cells. Our results indicate that pDCs have regulative functions and in conjunction with Treg cells they control inflammation in the gut during dysbiosis.

P.D1.02.17
Hyperinsulinemia following IFNgamma induced insulin resistance in skeletal muscle boosts the antiviral CD8 T cell response

***M. Šestan**[1], S. Marinović[1], I. Kavazović[1], D. Cekinović[2], S. Wueest[3], T. Turk Wensveen[4], I. Brizić[1], S. Jonjic[1], D. Konrad[3], F. M. Wensveen[1], B. Polić[1];*
[1]University of Rijeka; School of Medicine, Rijeka, Croatia, [2]Infectious diseases department, Clinical Hospital Center Rijeka, Rijeka, Croatia, [3]Division of Pediatric Endocrinology and Diabetology and Children's Research Centre, University Children's Hospital, Zurich, Switzerland, [4]Department of Endocrinology, Clinical Hospital Center Rijeka, Rijeka, Croatia.

Diabetes Mellitus type 2 (DM2) is a chronic metabolic disorder, defined by an inability of the pancreas to properly control blood glucose levels mainly because of systemic insulin resistance (IR). Patients with pre-diabetes may have years of IR without loss of glycemic control, followed by an abrupt increase of blood glucose levels beyond DM2 threshold values. Which event causes this acute loss of glycemic control is unknown. Here we investigated whether and how different viral infections (MCMV, LCMV, Influenza A) influence glucose homeostasis in lean and pre-diabetic obese subjects. We found that viral infections cause a transient systemic IR in lean mice and humans. Virally-induced IFNγ specifically targets skeletal muscle to downregulate the insulin receptor. In lean mice, infection does not cause loss of glycemic control because of increased production of insulin by the pancreatic β-cells. Activated CD8 T cells express the insulin receptor and in vitro activation of these cells in the presence of insulin enhances antiviral immunity through direct promotion of CD8 effector T cell function. Infection-induced hyperinsulinemia therefore boosted anti-viral CD8 T cell responses in vivo. However, in pre-diabetic mice with hepatic insulin resistance caused by diet-induced obesity, viral infection resulted in loss of glycemic control and aggravation of microvascular complications of DM2. Altogether, we identified a new immune-endocrine regulatory feed-back mechanism of antiviral immunity and provided valuable new insights in the underlying pathophysiology of DM2.

P.D1.02.18
Opposing effects of *A. muciniphila* and *C. rodentium* on autoimmune diabetes in NOD mice

***S. Silojärvi**[1], S. Pöysti[1], R. Toivonen[1], A. Hänninen[1,2];*
[1]Institute of Biomedicine, Turku, Finland, [2]Turku University Hospital, Hospital District of Southwest Finland, Turku, Finland.

Akkermansia muciniphila is a common symbiont in healthy gut of both humans and mice, while *Citrobacter rodentium* is a mouse pathobiont which promotes colitis in mice. Since gut microbiota is implicated in type 1 diabetes (T1D), the effects of these microbes were tested on diabetes and autoimmunity in the non-obese diabetic (NOD) mouse. While *A. muciniphila* delayed diabetes incidence compared with vehicle control, *C. rodentium* accelerated it in NOD mice. Although no significant differences were found in the amount of mucin, bacterial count or localization between colons of bacteria-administrated and control mice, lymph node fluorescence *in situ* hybridization (FISH) -stainings indicate that *C. rodentium* effects the emanation of bacterial DNA from colon to colon mesenteric and pancreatic lymph nodes. Colon microbiota also effects autoimmunity, which can be seen as differences in pathogen induced TLR2 and TLR4 -levels (Toll-like receptor) and in bacterial lipopolysaccharide (LPS) and interferon-gamma (IFN-γ) induced CXCL10-levels (C-X-C motif ligand) in pancreatic islets. Different levels of islet-invading white blood cells (insulitis) indicate that colon microbiota effects islet inflammation and beta-cell apoptosis. We conclude that the autoimmune process leading to islet-destruction in the NOD mouse is affected by gut microbiota and that it can be modified by oral administration of an isolated single symbiont or pathobiont.

P.D1.02.19
A potential link between age-related changes in the gut microbiome and changes in the germinal centre response of Peyer's patches

***M. Stebegg**, S. Innocentin, C. Gilbert, M. Linterman;*
Babraham Institute, Cambridge, United Kingdom.

The germinal centre (GC) response generates memory B cells and long-lived plasma cells that secrete high-affinity antibodies. In the gut, the GC response in Peyer's patches (PPs) is an important source of IgA-secreting plasma cells. IgA antibodies are secreted into the gut lumen where they have an important role in controlling the composition of the gut microbiome. In ageing, the GC response in lymph nodes has been shown to be impaired, leading to reduced antibody affinity maturation and memory responses. We hypothesised that an impaired GC response in PPs affects the affinity and quantity of secreted IgA antibodies and thereby drives some of the age-related changes in the gut microbiome. When analysing the GC response of Peyer's patches from aged C57BL/6 and Balb/c mice, we observed an age-related reduction in the proportion of GC B cells in 2 year old animals compared to 3 months old mice. Further, 16S-seq data from our ageing mouse colony confirmed changes in the gut microbiome of 2-year-old mice when compared to 3-month-old mice. While bacterial diversity was not affected, we observed a higher prevalence of *Bifidobacterales* in young mice, while the old microbiome was enriched for *Enterboacteriales*. Taken together, there is a correlation between a poor GC response and changes in the microbiome in ageing. Future work aims to determine if this is a causal relationship.

P.D1.02.20
The effect of *E. coli* O83:K24:H34 on human and murine dendritic cells

***L. Súkeníková**, P. Petrásková, J. Hrdý;*
Institute of Immunology and Microbiology, Prague, Czech Republic.

A balanced microbiome is greatly beneficial for a host. Any disturbance is associated with various problems and diseases, therefore, a number of experiments focus on correction for dysbiosis by probiotic supplementation. One of the promising probiotics appears to be *Escherichia coli* O83:K24:H31 (*E. coli* O83) but the exact mechanism of its positive effect has not yet described. To uncover it, we focused on differences between human and murine dendritic cells (DCs) after *E. coli* O83 stimulation.
Both human and murine DC-like cells were obtained by *in vitro* differentiation from progenitor cells and then stimulated with probiotics and LPS for 24 hours. The appearance of DC surface markers was analysed by flow cytometry and the relative gene expression (qPCR) and secretion (ELISA) of various cytokines and enzymes were tested.
The expression of DC activation markers CD80, CD86 and MHCII was significantly increased after stimulation by *E. coli* O83, indicating that DC-like cells are able to engulf and process probiotic antigens and reflecting their maturation state. Besides that, primed human DC-like cells produce higher amounts of IL-10 cytokine and express indole 2,3-dioxygenase enzyme. A slightly different mechanism occurs in the murine cells expressing also inducible nitric oxide synthase, typical for activated macrophages.
Together, results suggest that the positive effect of *E. coli* O83 is mediated by the reinforcement of tolerogenic DCs, thus supporting T regulatory cells which play a critical role in the induction of tolerance to self antigens and also to components of the microbiome.
Acknowledgment: AZV 15-26877A, SVV 260 369

P.D1.02.21

Proinflammatory microbial profiles associate with respiratory infections in older adults

*S. Fuentes[1], J. Ferreira[1], J. Pennings[1], G. den Hartog[1], J. van Beek[1], **D. van Baarle**[1,2];*
[1]National Institute for Public Health and the Environment, Bilthoven, Netherlands, [2]University medical Center Utrecht, Utrecht, Netherlands.

There is increasing evidence that interactions between the gut microbiome and the immune system can shape immune responses. With ageing, the immune response deteriorates leading to increased susceptibility to respiratory infections, posing a major health threat.
We studied the impact of gut microbiota composition on the development of respiratory infections using a cohort of older adults (=60 years) followed for influenza-like illness (ILI). Participants were sampled within 48-72hours of reporting an ILI (n 223), 7-9 weeks after ILI (n= 201) and compared to individuals without any ILI event (n=189).
Significant differences were found in the phyla Proteobacteria, Bacteroidetes and Firmicutes. Prediction analyses by Random Forest indicated the species *Ruminococcus torques* as top predictor for ILI. Stratification of groups by presence (Rt+) or absence (Rt-) of *R.torques* revealed higher abundance of pathogens from the *E.coli/Shigella* group. Furthermore, individuals from the Rt+ group showed significant lower diversity, a biomarker for gut microbiota resilience. Although levels of *R.torques* decrease after ILI recovery, levels of *E.coli/Shigella* remain elevated and beneficial bacterial groups (f.e.*Akkermansia*) remain reduced. As *R.torques* has been associated with a proinflammatory state, we measured inflammatory mediators including CRP, cytokines and chemokines. Redundancy analysis showed a proinflammatory profile in the ILI+Rt+ group both locally and systemically.
In conclusion, we reveal a proinflammatory microbiome profile in individuals presenting with ILI and identify potential microbial biomarkers for severity of disease. Understanding how gut microbiota influence onset and severity of respiratory infections can provide new leads for interventions targeted at microbiota modulation in the aging population.

P.D1.02.22

ROLE OF MICROBES IN HUMAN HEALTH, EFFECT ON INFECTIOUS DISEASE DYNAMICS

***M. A. YUSUF**;*
Osun State Polytechnic, Iree, Nigeria.

The outbreak infectious disease in human health can now be investigated to identify microbes or pathogen and carriers for control of infections. The process of this microbiome outbreak can be in stages which can be blocked by different defense mechanisms: host is exposed to infectious particle by an infected individual, the mode of transmission and stability of an infectious person outside the host determine its infectivity.some pathogens such human immunodeficiency virus {HIV} are spread only by the exchange of bodily fluids,early contact of the microbes with a new host occur through an epithelia surface,the skin or the internal mucosal surface of the respiratory,gastrointestinal and urogenital tracts.The gut microbes in disease-inflamation:microbial resident of the human gut are a major attributes in the development and maintenance of health but it differs from patient to patient.The causative agent fall into these: viruses,bacteria, fungi and protozoa.These approaches are also transforming our understanding of how interaction and focus between the human microbiota and the host in order to provide an overview of the microbial role in basic biological processes and in the development and progression of major human diseases such as infectious disease, gastrointestinal cancer.The purpose of this study is to check the role of microbes in health and disease and how the research can be applied to medicine and therapeutic target in clinical practice.

References

Falkow S {1988}: molecular kosh" postulate applied to pathogenetic ,Rev infect dis 10 s 274 5776
Genome medicine {2013} ;5:81
Charles and Janeway,Paul travers:Immunobiology;immune system in human health and disease, 5th edition

P.D1.03 Microbiome, metabolites and the immune system - Part 3

P.D1.03.01

The effector phenotype of human mucosal-associated invariant T (MAIT) cells in age-associated *Clostridium difficile* infections

***I. Bernal**[1,2], B. Bulitta[2], L. Gröbe[2], M. Neumann-Schaal[3,4], J. Hofmann[4], D. Jahn[4], A. Canbay[1], D. Bruder[1,2], L. Jänsch[2];*
[1]Otto-von-Guericke University of Magdeburg, Magdeburg, Germany, [2]Helmholtz Centre for Infection Research, Braunschweig, Germany, [3]Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, [4]Technical University Braunschweig, Braunschweig, Germany.

Clostridium difficile infection (CDI) can cause life-threating inflammatory responses in the intestinal mucosa and has become the nosocomial infection with the highest medical and economical relevance in Germany. However, our knowledge about host immunity in human CDI is fragmentary, and especially the role of memory effector T cells, like mucosal-associated invariant T (MAIT) cells, remains elusive. MAIT cells are innately pathogen-reactive and restricted by MHC class 1-related protein 1 (MR1), which presents antigenic bacterial metabolites of the riboflavin pathway. Presently the role of MAIT cells in CDI is unknown but interestingly their blood frequency decreases in age whereby the incidence of CDI increases. Thus, MAIT cells might mediate underscored protection against CDI infection and their functional impairment might increase susceptibility to CDI. We have recently reported the molecular effector phenotype of MAIT cells in healthy individuals (Bulitta et al., 2018) and here now elucidate their status und responsiveness in age-matched donor- and CDI-patient cohorts. As part of two consortia (ABINEP, CDIFF) and in cooperation with our clinicians we established an analytical pipeline allowing detailed molecular phenotyping with the support of proteomics. We will present results on the age-dependent alteration of MAIT cell effector functions, thereby complementing knowledge on the development and immunosenescence of the MAIT cell compartment. Beyond this we will present data characterizing the responsiveness of MAIT cells towards *C. difficile*. We defined the riboflavin synthesis of selected clinical isolates and using *ex vivo* isolated MAIT cells characterized which effector functions are activated dose- and MR1-dependently by *C. difficile*.

P.D1.03.02

Transcriptome profiling of Staphylococci-infected keratinocytes provides insight into the commensal-induced protective effect against *S. aureus*

***K. Bitschar**[1], L. Klink[1], B. Krismer[2], A. Peschel[2], B. Schittek[1];*
[1]Department of Dermatology, University Hospital Tübingen, Tübingen, Germany, [2]Interfaculty Institute of Microbiology and Infection Medicine, Infection Biology, University of Tübingen, Tübingen, Germany.

Introduction: Our skin is constantly exposed to a large number of pathogens while at the same time undergoing selective colonization by harmless commensal microorganisms such as *S. epidermidis*. We previously showed that secreted factors of *S. epidermidis* protect human and mouse skin against *S. aureus* infection. This work aims at elucidating the mechanism of this commensal-induced protective effect as well as providing a deeper understanding of how keratinocytes discriminate commensals from pathogens.
Materials and Methods: In order to identify the differential innate immune response triggered by commensals or pathogens we compared the transcriptomes of *S. epidermidis* and *S. aureus*-infected primary human keratinocytes by applying RNAseq. Furthermore, an *in vitro* skin infection model with keratinocytes and human skin explants as well as an *in vivo* epicutaneous mouse skin infection model were used to analyze the innate immune response induced by Staphylococci.
Results: We show that *S. epidermidis* is able to alarm keratinocytes by inducing the expression of Nf-κB target genes while at the same time preventing excessive inflammation. Hereby, the alarmin Interleukin-1α, which itself is sufficient to induce the protective effect, seems to play a key role. Consequently, the *S. epidermidis*- mediated protection is lost in IL-1R-deficient mice.
Conclusion: In healthy skin *S. epidermidis*, as part of the skin microbiota, alarms keratinocytes and thus creates a protective environment which prevents *S. aureus* from colonizing the skin. Further studies will provide deeper insight into the mechanisms of the IL-1R-mediated modulation of the innate immune response, which reduces *S. aureus* skin infection.

P.D1.03.03

Synergistic action of soluble-pattern recognition molecule pentraxin 3 (PTX3) with myeloperoxidase (MPO)-mediated bacteria killing

***K. Daigo**[1,2,3], D. Morone[2], S. Valentino[2], M. Sironi[2], F. Petroni[2], A. Doni[2], A. Inforzato[2], B. Bottazzi[2], A. Mantovani[2];*
[1]Nippon Medical School, Kanagawa, Japan, [2]IRCCS Humanitas Research Hospital, Milan, Italy, [3]The University of Tokyo, Tokyo, Japan.

Introduction: PTX3 is a soluble-pattern recognition molecule that plays non-redundant protective roles against infections through pathogens recognition, complement regulation and opsonization. PTX3 comprises eight-identical protomers, each consisting of a conserved C-terminal pentraxin domain and an N-terminal domain unrelated to other proteins. We previously reported that PTX3 interacts with MPO, a bactericidal enzyme in neutrophils. Both PTX3 and MPO have been reported as protective molecules against *Aspergillus fumigatus* infection. Aim of our study is to investigate the role of PTX-MPO interaction on *A. fumigatus* conidia killing. Results: Characterizing structural features of PTX3-MPO interaction, we found that PTX3 N-terminal domain was responsible for MPO binding. Surprisingly, MPO enzymatic activity was increased in the presence of PTX3, an effect recapitulated by the PTX3 N-terminal domain. Consistent with the effect, PTX3 enhanced MPO-mediated conidia killing and this action was mainly exerted by PTX3 N-terminal domain. In contrast, when we evaluate the influence of PTX3 to conidia-bound MPO, we observed that full-length of PTX3 had no effect, while PTX3 N-terminal domain maintains the capacity to enhance both enzymatic and conidia killing activity of MPO. Finally, preliminary data indicated that exogenous PTX3 can amplify MPO-mediated conidia killing in neutrophil extracellular traps (NETs) from human neutrophils. Conclusions: Our data show that PTX3, through its interaction with MPO, can amplify MPO-mediated conidia killing mainly through the N-terminal domain. This could represent a novel antimicrobial mechanism of PTX3. Further studies will be needed to define the impact of our observations on the anti-microbial effects exerted by PTX3 in neutrophils.

P.D1.03.04

Effects of silicon-rich water intake during chronic ingestion of aluminum on the systemic and peritoneal inflammation

T. Dzopalic[1], *Z. Radovanovic*[1], *B. Djindjic*[1], *A. Veljkovic*[1], *M. Dunjic*[2], *D. Krstic*[3], *N. Djindjic*[1], *B. Bozic Nedeljkovic*[4];
[1]*University of Nis, Medical Faculty, Nis, Serbia,* [2]*European University, Novi Sad, Serbia,* [3]*University of Nis, Faculty of Occupational Safety, Nis, Serbia,* [4]*University of Belgrade, Faculty of Biology, Belgrade, Serbia.*

Introduction: Ingestion of the aluminum by nutrients lead to its accumulation in human tissues, inducing various disorders. Silicon-rich water is a great source of bioavailable silicic acid, a natural antagonist of aluminum. The aim of study was to evaluate the role of silicon-rich water intake on the systemic inflammation and functional characteristics of peritoneal macrophages (PMs) of rats chronically exposed to low levels of aluminum. Materials and Methods: One month-old female Wistar Albino rats were administered aluminum chloride dissolved in distilled water (1.6 mg/kg body weight in 0.5 mL) by gavage for 90 days. The rats were then given standard (6 mg/L) or silicon-rich (19 mg/L silicon) water (n=7/group). Control rats underwent sham gavage and received standard or silicon-rich water (n=7/group). Blood was assessed for cytokine levels by ELISA. Unstimulated and lipopolysaccharide (LPS)-stimulated PMs were examined in terms of phagocytic activity and cytokine secretion. Results: Treatment with silicon-rich water did not change serum TNF-α levels in aluminum-treated animals compared to non-treated animals. Aluminum-intoxication increased serum IL-2 and this was reversed by silicon-rich water ingestion. LPS-stimulated PMs from aluminum-intoxicated rats exhibited low phagocytic activity and release of TNF-α, this was significantly improved by silicon-rich water intake. The presence of silicon-rich water induced significant production of IL-10 by LPS-stimulated and unstimulated PMs of aluminum-exposed rats. Conclusions: Chronic ingestion of aluminum initiated systemic and peritoneal inflammation, while silicon-rich water restored levels of IL-10 and TNF-α produced by PMs thus preventing prolonged inflammation. Finally, silicon intake can decrease the immunotoxicity of aluminum.

P.D1.03.05

High protein diet influneces the severity of colitis in mice

A. Fajstova*, K. Klimesova, N. Galanova, S. Coufal, Z. Stehlikova, M. Kostovcik, M. Buganova, H. Tlaskalova-Hogenova, M. Kverka;*
Institute of Microbiology of the CAS, Prague, Czech Republic.

Introduction: Gut microbiome composition is strongly influenced by our diet, which also influences immune response. The aim of our experiments was to examine the association of diet, microbiome and immune response with Inflamatory bowel diseases development. Methods: We kept conventionaly (CV) reared and germ-free (GF) BALB/c mice and RAG2-/- mice on BALB/c background on High protein diet (HPD) or Control diet (CTRL) for 2-3 weeks. Then, we used 3% Dextrane Sodium Sulfate in drinking water to induce acute colitis. After 5 days, we terminated the experiment, evaluated the severity of colitis and collected samples. Results: We found that acute colitis was more severe in HPD fed CV mice compared to CTRL. HPD fed mice had shorter colon, higher disease activity index (calculated from weight loss, bleeding and diarrhea), greater weight loss and histological evaluation of colon samples showed more damage of the tissue. Similarly, in immunodeficient RAG2-/-mice, colitis was more severe in mice fed with HPD. Although there were no significant differences in general microbiome composition between mice on HPD versus CTRL diet, gut microbiota could have a role in the colitis development. Interestingly, in GF mice, acute colitis was equally severe on both diets. As expected, we found significant differences in metabolites pattern in feces of CV versus GF mice which may suggest that the effect of microbiota is probably mediated through metabolite production which can influence immune system activity. This work has been supported by grant 16-06326S from Czech Science Foundation.

P.D1.03.06

Deciphering the microbiome and metabolic factors contributing to protection against ulcerative colitis

J. Gaifem[1,2], *M. Garzón*[3], *C. Ubeda*[3,4], *L. Gonçalves*[5], *A. Longatto-Filho*[1,2,6], *A. Belinha*[1,2], *I. Mesquita*[1,2], *E. Torrado*[1,2], *C. Cunha*[1,2], *A. Carvalho*[1,2], *F. Rodrigues*[1,2], *M. Saraiva*[7,8], *A. Castro*[1,2], *R. Silvestre*[1,2];
[1]*Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal,* [2]*ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal,* [3]*Departamento de Genómica y Salud, Centro Superior de Investigación en Salud Pública – FISABIO, Valencia, Spain,* [4]*Centers of Biomedical Research Network (CIBER) in Epidemiology and Public Health, Madrid, Spain,* [5]*Instituto de Tecnologia Química e Biológica (ITQB) António Xavier, Universidade NOVA, Oeiras, Portugal,* [6]*Molecular Oncology Research Center, Barretos Cancer Hospital, São Paulo, Brazil,* [7]*i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal,* [8]*IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal.*

Introduction: Inflammatory bowel disease is an immune-mediated disorder triggered by environmental factors affecting the mucosal barrier and the gut microbiota balance. While analyzing DSS-induced colitis in genetically similar C57BL/6 mice housed in two different animal facilities, we serendipitously observed a group of animals with a remarkable resistance to disease development. We aim to identify the microbial organisms/metabolites responsible for the protective phenotype.
Methods: C57BL/6 mice were administered with 3% DSS and disease activity index was scored. Colonic histological evaluation was performed by Hematoxylin-Eosin. Stool samples were analyzed by metagenomics while colon/serum metabolites were screened by NMR. Intestinal epithelial barrier function and permeability were evaluated by qPCR and FITC-dextran translocation.
Results: The two groups of mice display distinct microbiome and metabolic profiles, clustering separately in multivariate data analysis. We identified 2 bacterial species that are statistically enriched in resistant mice. These can be at the genesis of the protective phenotype, since fecal microbiota transplant from resistant to susceptible mice was able to reverse the susceptibility to colitis. From the 37 metabolites quantified by NMR, 12 are significantly increased in resistant mice. Moreover, our data shows significantly increased levels of IL-10, IL-17 and IL-22, as well as increased expression of claudin- and mucin-encoding genes in resistant mice prior to colitis induction.
Conclusions: Our results suggest that resistant mice display an intestinal epithelial barrier more prone to sustain an inflammatory insult. We are currently modulating the microbiome with putative microbiota/metabolic candidates to explore which mechanisms are contributing for protection to intestinal inflammation.

P.D1.03.07

L-Threonine supplementation during colitis induction impairs goblet cell number and delays disease recovery

J. Gaifem[1,2], *L. Gonçalves*[3], *R. Dinis-Oliveira*[4,5,6], *C. Cunha*[1,2], *A. Carvalho*[1,2], *E. Torrado*[1,2], *F. Rodrigues*[1,2], *M. Saraiva*[7,8], *A. Castro*[1,2], *R. Silvestre*[1,2];
[1]*Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal,* [2]*ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal,* [3]*Instituto de Tecnologia Química e Biológica (ITQB) António Xavier, Universidade NOVA, Oeiras, Portugal,* [4]*IINFACTS – Institute of Research and Advanced Training in Health Sciences and Technologies, Department of Sciences, University Institute of Health Sciences (IUCS), CESPU, CRL, Gandra, Portugal,* [5]*UCIBIO, REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal,* [6]*Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, Porto, Portugal,* [7]*i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal,* [8]*IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal.*

Introduction: Dietary nutrients have emerged as potential therapeutic adjuncts for inflammatory bowel disease (IBD) given their impact on intestinal homeostasis through the modulation of immune response, gut microbiota composition and epithelial barrier stability. Several nutrients have already been associated with a protective phenotype. Yet, there is a lack of knowledge towards the most promising ones as well as the most adequate phase of action.
Methods: C57BL/6 mice were administered with 2% DSS. The colon metabolic profile was assessed by NMR. Alcian Blue/PAS was performed for goblet cell counting. Cytokine levels were measured by ELISA.
Results: We have observed a 2-fold decrease in threonine levels in mice subjected to colitis. We assessed the effect of threonine supplementation in the beginning of the inflammatory process (DSS+Thr) or when inflammation is already established (DSS+Thr D8). Colitis progression was similar between treated groups and control colitic mice, yet threonine had a surprisingly detrimental effect when administered in the beginning of the disease, with mice displaying a delayed recovery when compared to control mice and DSS+Thr D8 mice. Although no major changes were found in their metabolic profile, DSS+Thr mice displayed altered expression in mucin-encoding genes and in goblet cell counts, unveiling an impaired ability to produce mucus. Moreover, IL-22 secretion was decreased in DSS+Thr mice when compared to DSS+Thr D8 mice.
Conclusion: These results suggest that supplementation with threonine during colitis induction impact goblet cell number and delays the recovery period. This reinforces the importance of a deeper understanding regarding threonine supplementation in IBD.

P.D1.03.08

Comparative genomics and *in silico* epitope prediction for the development of specific monoclonal antibodies for microorganisms in drinking water

M. Göthel[1], *S. Hoppe*[2], *M. von Nickisch-Rosenegk*[2], *K. Hanack*[1];
[1]*University Potsdam, Department of Biochemistry and Biology, Immunotechnology group, Potsdam, Germany,* [2]*Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalytics and Bioprocesses (IZI-BB), Department of Bioanalytics and Biosensorics, Potsdam, Germany.*

Introduction: Contamination of drinking water by pathogenic microorganisms is a great concern worldwide and leads to severe infections in humans. So far, the diagnostic detection of these pathogens is mainly performed by microbiological methods such as cultivating the sample on agar plates and counting of the colonies. Flow cytometry and cell enzyme-linked immunosorbent assay (ELISA) are alternative methods to improve the monitoring of water quality. Therefore, specific monoclonal antibodies are indispensable for these techniques.

Methods: In this study, we performed a comparative genomics analysis based on next generation sequencing data of 50 microbial genomes to identify *E.coli* specific antigens useful as potential antibody targets. The genes presented in 30 different pathogenic *E.coli* strains but absent in 20 nonpathogenic *E. coli*/non-*E.coli* strains were subjected to an *in silico* analysis to identify secreted or surface-expressed proteins.
Results: From 226.735 genes we obtained a total of 10 genes which encode potential protein candidates. We analyzed the resulting proteins for linear epitope prediction as well as epitope surface accessibility and antigenicity. The epitopes with the highest scores to harbor specific epitopes have been cloned into a viral carrier protein used for immunization. With this approach we generated monoclonal antibodies specific to pathogenic *E.coli,* which could be part of a diagnostic tool for the detection of drinking water contaminations.
Conclusions: The described bioinformatic approach is able to identify novel epitope candidates for the generation of specific monoclonal antibodies specific to pathogenic microorganisms.

P.D1.03.09
Innate Recognition and Inflammasome Activation in Human Myeloid Immune Cells by Methanogenic Archaea

*T. Vierbuchen[1], C. Bang[2], R. Schmitz[3], **H. Heine**[1];*
[1]Research Center Borstel, Div. of Innate Immunity, Borstel, Germany, [2]University Hospital Schleswig-Holstein, Institute of Clinical Molecular Biology (IKMB), Kiel, Germany, [3]Kiel University, Institute for General Microbiology, Kiel, Germany.

The importance of the microbiota on health and immune homeostasis is widely accepted and the interaction between the microbiota and our body is currently investigated. However, most of these studies are focusing on bacteria alone, although viruses, fungi and archaea are also part of this microbial community. Recent studies showed that archaea are present at nearly every part of the body but their contribution to health and disease is not understood. It is known that the gut-associated methanogenic archaeon *Methanosphaera stadtmanae* induces inflammatory responses, however, the mechanism how this archaeon is sensed by the immune system has not been evaluated until now. This study aims to elucidate the receptors, archaeal structures and signaling pathways that are engaged upon activation of human immune cells by *M. stadtmanae*.
We show that *M. stadtmanae* induces secretion of pro-inflammatory cytokines as well as expression of type I and III interferons in primary human myeloid cells as well as in the monocytic cell line BLaER1. CRISPR/Cas9 generated KO cells were used to identify TLR7 and TLR8 as main receptors for recognition of *M. stadtmanae*. Furthermore, the archaeon induces a TLR8-dependent activation of the NLRP3 inflammasome sharing features with the LPS-induced alternative pathway.
For the first time, our results describe specific recognition of an archaeon by human immune cells in detail. As some studies indicate a potential connection of *M. stadtmanae* to inflammatory bowel disease and lung hypersensitivity, our findings might help to understand how archaea are involved in inflammatory diseases.

P.D1.03.10
The antigen specificity of resident memory T cells in the human lungs

*A. Oja[1], F. Morgana[1], B. Bardoel[2], D. van der Zwan[1], J. Mok[3], M. Maas[3], G. Brasser[3], R. van Lier[1], W. van Esch[3], **P. Hombrink**[1];*
[1]Department of Hematopoiesis, Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands, [2]Bacterial Infections and Immunity, University Medical Center Utrecht, Utrecht, Netherlands, [3]Department of Reagents, Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands.

Resident memory T-cells (TRM) are critical for local adaptive immune responses. In the lungs TRM are elementary for protection against airborne pathogens. This protection is superior relative to that provided by circulating T-cells and is modulated by the release of cytotoxic molecules. As TRM are in disequilibrium with circulation and most antigen discovery studies rely on peripheral blood for readout, little is known about the magnitude, phenotype and specificity of protective T-cell populations in the lungs. In this study we simultaneously dissect the phenotype and function of CD4+ and CD8+ T-cells in human lung tissue and compare these to the blood compartment. By using extensive HLA-tetramer combinatorial coding screens we analyzed the phenotype and frequency of lung CD8+ T-cells specific for respiratory and systemic viruses in donors with no recent history of infection. We demonstrate an enrichment of respiratory specific T-cells in the lung CD8+ T-cell compartment. While *Influenza* and *RSV* reactive CD8+ T-cells are biased to a CD103+ TRM phenotype, those specific for *CMV* and *EBV* are phenotypically identical to circulating cells. In contrast, influenza reactive CD4+ T-cells in the lungs lack expression of CD103, suggesting a strict spatial regulation. In addition we mapped the lung CD4+ T-cell response to the human pathogens *Staphylococcus aureus* and *pneumonia* using libraries of recombinant proteins and discovered novel immunodominant responses. The ability to monitor antigen-specific T-cell responses in human lungs, will facilitate subsequent translation into vaccination strategies that aim to boost local protection to airborne pathogens.

P.D1.03.11
MAIT cells are recruited to the intervillous space of the placenta by placenta-derived chemokines

*M. Solders[1], L. Gorchs[1], E. Tiblad[2,3], S. Gidlöf[2,3,4], E. Leeansyah[5,6], J. Dias[5], J. K. Sandberg[5], I. Magalhaes[7], A. Lundell[8], **H. Kaipe**[1,9];*
[1]Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden, [2]Department of CLINTEC, Karolinska Institutet, Stockholm, Sweden, [3]Center for Fetal Medicine, Karolinska University Hospital, Stockholm, Sweden, [4]Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden, [5]Department of Medicine, Huddinge, Karolinska Institutet, Stockholm, Sweden, [6]Program in Emerging Infectious Diseases, Duke-National University of Singapore Medical School, Singapore, Singapore, [7]Department of Oncology/Pathology, Karolinska Institutet, Stockholm, Sweden, [8]Rheumatology and Inflammation Research, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [9]Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden.

The intervillous space of the placenta is a part of the fetal-maternal interface, where maternal blood enters to provide nutrients and gas exchange. Little is known about the maternal immune cells at this site, which are in direct contact with fetal tissues. We have characterized the immune cell composition and chemokine profile in paired intervillous and peripheral blood from healthy mothers giving birth at term. We found that mucosal-associated invariant T (MAIT) cells were enriched in the intervillous blood compared to peripheral blood. Pregnant women had lower proportions of peripheral MAIT cells compared to non-pregnant women, further indicating that MAIT cells home to the placenta during pregnancy. We observed that the levels of several chemokines were dramatically higher in intervillous compared to peripheral blood, including MIF, CXCL10, and CCL25, whereas others were lower. Migration assays showed that MAIT cells migrate towards conditioned medium from placental explants. A multivariate discriminant analysis indicated that high levels of MIF and CCL25 were associated with high proportions of MAIT cells in intervillous blood. A positive correlation was observed between MAIT cell proportions and MIF levels in intervillous, but not in peripheral blood. Blocking of MIF or a combination of MIF, CCL25, and CCL20 in migration assays decreased the number of migrating MAIT cells. Finally, MAIT cells showed migratory capacities towards recombinant MIF. Together, these findings indicate that term placental tissues attract MAIT cells, and that this is partly mediated by MIF. The importance of MAIT cells in pregnancy remains to be determined.

P.D1.03.12
Experimental high-fat diet consumption leads to intestinal barrier damage and severe colitis

***K. Klimesova**, N. Galanova, A. Fajstova, S. Coufal, M. Kostovcik, M. Buganova, H. Tlaskalova-Hogenova, M. Kverka;*
Institute of Microbiology CAS, Prague, Czech Republic.

Diet is important environmental factor influencing gut homeostasis. Increased fat intake has been related to higher risk of inflammatory bowel disease development. Here, we focused on effects of high-fat diet consumption on colitis development in mouse model. Fourteen days before colitis induction, we transferred mice to diet with increased or normal fat content - high-fat diet (HFD, 22% fat) or control diet (CD, 5% fat), respectively. Then, we induced colitis with 3% dextran sodium sulfate (DSS) in drinking water for 7 days. Before DSS treatment, mice consuming HFD gained approximately twice more weight than CD mice and they have also significantly longer colon and bigger spleen. Moreover, HFD mice showed increased permeability of intestine measured by fluorescein isothiocyanate-conjugated dextran and mild inflammatory changes observed by microscopical analysis of hematoxylin/eosin stained sections. DSS treatment in HFD mice led to significant reduction of their body weight, diarrhea and rectal bleeding. The length of colon did not change significantly but spleen weight doubled compared to CD mice. In spleen of HFD mice, we found significant increase in Th17 cells - CD4+RorgT + population measured by flow cytometry, and higher production of IL-17 after CD3/CD28 stimulation. In conclusion, we confirmed that HFD significantly increased the sensitivity of mice to DSS colitis. Mice consuming HFD developed low local and systemic inflammation which could lead to mucosal immune system exhaustion and induction of systemic Th17 response. The study has been supported by Czech Science Foundation grants no. 16-06326S and 17-06632Y.

P.D1.03.13
Polyunsaturated fatty acids (PUFAs) dampen the inflammatory antiviral immune response by diminishing two dimensional (2D) antigen recognition by T cells

***E. M. Kolawole**, B. D. Evavold;*
University of Utah, Salt Lake City, United States.

Polyunsaturated fatty acids (PUFAs) have been shown to dramatically influence inflammatory responses. Although omega-3 fatty acids lead to the production of less inflammatory metabolites, little is known about how PUFAs change surface protein interactions and T cell activation. To probe the protein interactions, we utilized novel 2D based technologies for single cell and single molecule analyses of T cell receptor (TCR) engagement with peptide-MHC (pMHC) antigen. We found that dietary fish oil decreased 2D affinity of T cell receptor transgenic and polyclonal T cell responses in both CD8 and CD4 T cells at peak anti-viral immunity to lymphocytic choriomeningitis (LCMV) infection. In addition, an omega-3 based diet manipulated structural lipid content in plasma membranes leading to a reduced T cell frequency and changes in the prevalence of immunodominant epitopes. This reduced affinity led to decreased markers of activation and effector function. These data indicate that a major effect of dietary omega-3 fatty acids on T cells is the modification of the cell membrane to dampen the initial recognition of antigen.

P.D1.03.14

the dietary food additive maltodextrin promotes endoplasmic stress-driven mucus depletion and exacerbates intestinal inflammation

F. Laudisi[1], *D. Di Fusco*[1], *V. Dinallo*[1], *C. Stolfi*[1], *A. Di Grazia*[1], *I. Marafini*[1], *A. Colantoni*[1], *A. Ortenzi*[1], *C. Alteri*[1], *F. Guerrieri*[2], *F. Ceccherini-Silberstein*[1], *M. Federici*[1], *T. T. MacDonald*[3], *I. Monteleone*[1], *G. Monteleone*[1];
[1]*University of Rome Tor Vergata, Rome, Italy,* [2]*Istituto Italiano di Tecnologia, Rome, Italy,* [3]*Queen Mary University of London, London, United Kingdom.*

Food additives, such as emulsifiers, stabilizers or bulking agents, are present in the western diet and their consumption is increasing. However little is known about their potential effects on intestinal homeostasis. In this study we examined the effect of some of these food additives on gut inflammation. Maltodextrin (MDX)-enriched diet exacerbated intestinal inflammation in experimental models of colitis and ileitis. Analysis of the mechanisms underlying the detrimental effect of MDX revealed up-regulation of the inositol-requiring enzyme (IRE)1β, a sensor of endoplasmic reticulum (ER) stress, in goblet cells and reduction of mucin-2 expression with no significant change in mucosa-associated microbiota composition. Stimulation of murine intestinal crypts and the human mucus-secreting cells with MDX induced IRE1β via a p38 MAP kinase-dependent mechanism. Treatment of mice with the ER stress inhibitor Tauroursodeoxycholic acid prevented mucin-2 depletion and attenuated colitis in MDX-fed mice. Interestingly, mice receiving a prolonged MDX-enriched diet exhibited low-grade intestinal inflammation, which was characterized by focal inflammatory infiltrates, distortion of gland architecture and edema. In conclusion, this study shows, for the first time, that MDX-enriched diet triggers ER stress in goblet cells with consequent reduction of the intestinal content of mucin-2, thus making the host more sensitive to colitogenic stimuli. Our data supports the hypothesis that western diet rich in the food additive MDX can contribute to gut disease susceptibility.

P.D1.03.15

Identification of perturbed immune responses at the transcriptional and metabolic levels during Tuberculosis disease

A. Llibre[1,2], *E. Nemes*[3], *V. Rouilly*[1], *C. Posseme*[1,2], *S. Mabwe*[3], *B. Charbit*[4], *H. Mulenga*[3], *M. Musvosvi*[3], *S. Thomas*[1,2], *N. Bilek*[3], *T. J. Scriba*[3], *M. L. Albert*[2,4,5], *D. Duffy*[1,2,4], *Milieu Intérieur Consortium;*
[1]*Immunobiology of Dendritic Cells, Institut Pasteur, Paris, France,* [2]*Inserm U1223, Institut Pasteur, Paris, France,* [3]*South African Tuberculosis Vaccine Initiative, Division of Immunology, University of Cape Town, Cape Town, South Africa,* [4]*Centre for Translational Research, Paris, France,* [5]*Genentech Inc, San Francisco, United States.*

One quarter of the world's population is estimated to be latently infected with tuberculosis (TB). Adherence to treatment and emergence of multidrug resistance strains are key challenges for achieving TB eradication. Thus, new treatments are urgently needed and host targeted therapies offer great potential.
IFN-g (type II IFN) plays an essential role in the immune control of TB, however, the role of type I IFNs in M. tuberculosis (M. tb) infection remains controversial. It has also been reported that a shift from oxidative phosphorylation to aerobic glycolysis occurs in M. tb infected macrophages. We wish to understand the dynamics between IFN and cellular metabolism; bringing new insights into TB pathogenesis that may lead to the development of novel immunotherapies, targeting both immune and/or metabolic host cell pathways. Using M. tb antigens, we stimulated whole blood using the TruCulture system from 25 active TB patients (pre and post treatment) and 25 asymptomatic individuals with M. tb infection. Protein, metabolomic and gene expression analysis of more than 600 genes was performed using Luminex multi-analyte profiling, Mass Spectroscopy and Nanostring technologies, respectively.
We observed at baseline a high type I IFN transcriptional signature, a perturbed glycolysis pathway and diminished expression of MHC-II related genes in active TB patients as compared to controls. We detected stimuli-specific differences between asymptomatic controls and active cases, both at gene expression and metabolomic levels. Many of these perturbed phenotypes were restored to control levels after successful treatment suggesting that they may serve as on treatment prognostic biomarkers.

P.D1.03.16

Immunoglobulin G coating shows earlier microbiota-host interaction in MCJ-deficient model during murine experimental colitis

A. Peña Cearra[1], *M. A. Pascual-Itoiz*[2], *A. Fullaondo*[3], *J. Anguita*[2], *L. Abecia*[2];
[1]*CIC bioGUNE and University of the Basque Country, Derio, Spain,* [2]*CIC bioGUNE, Derio, Spain,* [3]*University of the Basque Country, Leioa, Spain.*

Introduction: Ulcerative Colitis (UC) is a relapsing disorder of the gastrointestinal tract characterized by intestinal inflammation and epithelial injury. Although, the precise cause of UC remains unknown, microbial-host interactions and mitochondrial dysfunction play a critical function. Therefore, it is needed to know the role that a natural negative regulator of mitochondrial respiration such as Methylation-controlled J protein (MCJ) exerts in the disease.
Materials and Methods: To analyze host-microbiota interaction in a Dextran Sodium Sulfate (DSS)-mediated colitis and antibiotic-mediated dysbiosis model, wild type and MCJ-deficient mice were used. Dysbiosis was promoted administrating orally a broad spectrum antibiotic, twice per day during 7 days. Later, colitis was induced by adding 3% (w/v) DSS (36-50 KDa) in the drinking water for 6 days followed by a 2 day recovery period. Flow cytometry was used to quantify IgG coated fecal bacteria. GraphPad software was used for statistical analysis.
Results: After 7 days of antibiotic administration, dysbiotic mice presented higher percentage of IgG coated bacteria compared to non dysbiotic groups. At day 6, dysbiotic non colitis mice reached basal IgG levels. However, after 6 days of DSS administration, colitis mice showed higher IgG levels except for non dysbiotic control mice that took until day 8.
Conclusion: Antibiotic administration affected IgG coating bacteria. Basal levels were recovered in 6 days, indicating the period that hosts immune system needs to achieve bacterial tolerance. However, higher IgG coating in MCJ deficient non dysbiotic colitis group indicated earlier microbial-host cross-talk due to the lack of MCJ.

P.D1.03.17

Lifelong asymptomatic infections: The role of the microbiota

R. Romero[1], *A. Zarzycka*[1], *A. Visekruna*[1], *F. Fischer*[1], *G. Frankel*[2], *B. Stecher*[3], *U. Steinhoff*[1];
[1]*Institute for medical microbiology and clinical hygiene, Marburg, Germany,* [2]*Imperial College London, London, United Kingdom,* [3]*Max von Pettenkofer-Institut, Munich, Germany.*

Introduction: *Citrobacter rodentium* is an enteric murine pathogen that is suitable to model lifelong asymptomatic infections of humans with the enteropathogenic *E. coli* (EPEC). It is still unknown why some individuals become lifelong asymptomatic carries of EPEC and unknowingly spread the disease, while others can develop an acute, in some cases life-threatening, clinical manifestation. This immunological situation can be mimiked in germ-free (GF) mice, as these animals are unable to eliminate *C. rodentium* and become lifelong asymptomatic carriers of the bacterium.
Material and methods: Bacterial communities essential for the elimination of *C. rodentium* were assessed by analysis of the microbiota composition. 16S rDNA sequencing, MALDI and FACS analysis were performed to analyze microbial populations. Immunological analyses of *C. rodentium* infected mice were performed by FACS, qPCR and histology.
Results: Conventionalization of GF-mice reverses the asymptomatic carrier state by mediating activation and migration of neutrophils into the intestine. Interestingly, colonization of GF mice with 12 bacteria representing the five most prevalent phyla of the intestinal microbiome is insufficient to induce clearance. This shows that distinct bacterial species are able to trigger a proper immune response that results in sterile clearance of the pathogen. Conclusions: Eradication of asymptomatic infections with enteropathogens is mediated by the activation of immune cells through specific commensals. Surprisingly, dominant bacteria representing the microbial phyla can not compensate for the lack of these specifc bacteria. These results may explain why humans despite harbouring a complex microflora may become aysmptomatic carriers.

P.D1.03.18

Inflammatory monocytes promote bacterial resistance to antibiotic killing during influenza and*Staphylococcus aureus* coinfection

K. J. Fischer, V. Kumar Yajjala, C. Bauer, S. Bansal, R. Chen, ***K. Sun****;*
University of Nebraska Medical Center, Omaha, United States.

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a significant contributor to morbidity and mortality during recent influenza pandemics and epidemics. The mechanism responsible for this influenza-induced susceptibility to secondary bacterial infection is incompletely understood. We have previously shown in a clinical-relevant mouse model that preceding influenza infection suppresses the effectiveness of antibiotics for treatment of secondary *S. aureus* infection. This suppression coincides with influenza-induced accumulation of inflammatory monocytes. C-C chemokine receptor type 2 (CCR2) is responsible for pulmonary monocyte recruitment after influenza infection. Here we demonstrate that compared with wild-type animals, CCR2-deficient ($Ccr2^{-/-}$) mice are more resistant to post-influenza *S. aureus* infection, despite a delay in viral clearance. In agreement with their increased survival rate, influenza/MRSA co-infected $Ccr2^{-/-}$ mice exhibit a significantly improved ability in lung bacterial clearance, with or without antibiotic treatment. Mechanistically, our results suggest that influenza-induced inflammatory monocytes serve as reservoirs to protect bacteria from killing by antibiotics and more potent neutrophils, thereby promoting lung bacterial persistence during secondary *S. aureus* infection. Collectively, we demonstrate in this study that inflammatory monocytes constitute an important and hitherto underappreciated mechanism of the conflicting immune requirements for viral and bacterial control in the lung, which subsequently leads to exacerbated outcomes of influenza and *S. aureus* coinfection. (This work was supported by National Institutes of Health/National Heart, Lung, and Blood Institute R01 HL118408 to K.S.)

P.D1.03.19

Vgamma9Vdelta2 T cells proliferate in response to phosphoantigens released from erythrocytes infected with asexual and gametocyte stage *Plasmodium falciparum*

C. Liu[1], *N. Emami*[2], *J. Pettersson*[3], *L. Ranford-Cartwright*[4], *I. Faye*[2], *I. Parmryd*[1];
[1]*Medical cell biology Uppsala univerisity, Uppsala, Sweden,* [2]*Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden,* [3]*Department of Chemistry, Uppsala University, Uppsala, Sweden,* [4]*Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom.*

Introduction:Vγ9Vδ2 T cells are the dominant γδ T cell subset in human peripheral blood. Vγ9Vδ2 T cells are activated by phosphoantigens, primarily isoprenoid pyrophosphates. It has long been known that phosphoantigens that stimulate Vγ9Vδ2 T cells are released from *Plasmodium falciparum*-infected erythrocytes upon schizont rupture, but recently it was reported that release also occurs at the ring stage of the parasite life cycle. We therefore set out to investigate whether phosphoantigens are released at any other of the blood stages. **Basic Method:** Pure cultures of *Plasmodium falciparum*-infected erythrocytes at all asexual stages were obtained using a combination of a plasmion and sorbitol synchronization and of sexual gametocytes by using heparin. Sterile-filtered media from the cultures was assessed for its ability to cause proliferation of Vδ2 T cells from human PBMCs. **Results**:Vγ9Vδ2 T cells were proliferated by addition of the media from erythrocytes infected with parasites at all blood stages (ring, trophozoite, schizont, rupturing schizont and gametocytes). The proliferation was caused by phosphoantigens, verified by phosphatase-treatment. The iron-levels in the media from infected cultures were not elevated indicating that erythrocyte rupture was minimal. **Conclusion**: Phosphoantigens in sufficient quantities to stimulate Vδ2 T proliferation are released by intact parasite-infected erythrocytes at all blood-stages of the parasite life cycle. **Grants**: This work was supported by grants from INFRAVEC (EU/FP7) to IF and the Swedish Research Council, AFA Insurance and Claes Groschinsky's Foundation to IP.

P.D1.03.20

Assessment of multivalent viral vectored vaccines against outbreak pathogens: Ebola, Marburg & Lassa

A. Flaxman[1], *S. Sebastian*[1], *C. Gilbride*[1], *H. Sharpe*[1], *S. Dowall*[2], *S. Charlton*[2], *J. Purushotham*[1], *A. Hill*[1], *S. Gilbert*[1], *T. Lambe*[1];
[1]*Jenner Institute, University of Oxford, Oxford, United Kingdom,* [2]*Public Health England, Salisbury, United Kingdom.*

Since the 2013-2016 Ebolavirus outbreak, there have been a number of other documented outbreaks of lethal haemorrhagic fever caused by filoviruses and arenaviruses. Filovirus family members including Ebolavirus and Marburg virus and arenaviruses such as Lassa virus cause haemorrhagic fevers with high mortality rates in humans. As yet, no specific treatment or prophylactic multivalent vaccine against filoviruses or arenaviruses has been licensed. It is generally accepted that either a mixture of monovalent vaccines or, preferably, a multivalent vaccine, will be required to confer protective immunity against viral haemorrhagic fever. The costs of developing individual vaccines against filoviruses and an arenavirus (Lassa virus (LASV)) may be prohibitively high. Considering the geographical overlap between Ebola, Marburg and Lassa virus endemic areas, a multivalent vaccine would be of significant benefit. Our pre-developed core vaccine platforms (adenoviral vectors and MVA) can express multiple antigens and have demonstrated capability for the induction of durable immune responses in other infectious disease settings e.g. influenza/malaria.
We have now used this technology to develop multivalent vaccines against several lethal haemorrhagic fever- causing viruses, including Filoviruses (Ebolaviruses & Marburg virus) and Arenaviruses (Lassa virus). These vectors elicited antigen expression *in vitro* and were immunogenic *in vivo*. We assessed different vaccination regimes in mice and tested efficacy by challenging guinea pigs with Ebola Zaire post-vaccination. Our viral vectored vaccines showed protection from disease in this model. Therefore, we have successfully developed preclinical vaccine candidates against three outbreak pathogens (Ebola, Marburg and Lassa) using a multivalent platform.

P.D1.03.21

omplex and unusual antigen-specific activation ofVδ2+ gamma-delta T-cells by the BTN3A-receptor complex.

D. A. Rhodes[1], *S. Smith*[1], *J. Trowsdale*[1], *N. McCarthy*[2], *M. Eberl*[3];
[1]*University of Cambridge, Cambridge, United Kingdom,* [2]*Centre for Immunobiology, Blizard Institute, London, United Kingdom,* [3]*Systems Immunity Research Institute, Cardiff University, Cardiff, United Kingdom.*

The gamma delta T-cell pool in human blood is dominated by Vγ9/Vδ2 (Vδ2+) T-cells, which are specialised to detect phosphoantigens (pAg) produced by microbes and tumours. Activation of Vδ2+ T-cells by pAg HMB-PP and IPP requires expression of BTN3A molecules by presenting cells. At present it is not clear what conformation of the three BTN3A isoforms transmits activation signals nor how they are regulated. To investigate this, we used BTN3A knockout HeLa cells (HeLa/sgBTN3A), generated by CRISPR/cas9 gene editing, together with stable re-expression of BTN3A isoforms in all combinations. BTN3A1 and BTN3A3 proteins were subject to cell intrinsic suppression, whereas BTN3A2 was expressed constitutively. In co-culture experiments with expanded Vδ2+ T-cells, we confirmed that BTN3A1 was necessary but not sufficient to transmit activation signals, with BTN3A2 and/or BTN3A3 also being required. There were differences in responses induced by activating stimuli, HMB-PP, IPP and agonist antibody CD277 20.1. The combination of BTN3A1 with BTN3A3 induced more potent cytotoxicity and cytokine production as measured by lactate dehydrogenase release and IFN-γ secretion into co-culture medium, whereas the BTN3A2/BTN3A3 combination was non-functional by these stimuli. A critical role for the BTN3A1 and BTN3A2 juxta-membrane domains, pivotal in shaping the BTN3A-dependent activating epitope required for γδ T cell receptor engagement, was shown. Our results reveal additional complexity and highlight the unusual nature of the antigen-specific activation of human Vδ2+ T-cells, which is regulated by cell intrinsic mechanisms linked to infection and cell transformation by pAg sensing and control of BTN3A protein stability.

P.D1.04 Microbiome, metabolites and the immune system - Part 4

P.D1.04.01

***Anaeroplasma* of the phylum *Tenericutes* induce mucosal TGF-â and promote IgA production**

A. Beller[1], *A. Kruglov*[1,2], *P. Durek*[1], *K. Werner*[1], *V. von Goetze*[1], *J. Ninnemann*[1], *K. Lehmann*[1], *B. Siegmund*[3], *U. Hoffmann*[1], *G. A. Heinz*[1], *M. F. Mashreghi*[1], *A. Radbruch*[1], *H. D. Chang*[1];
[1]*German Rheumatism Research Center (DRFZ), a Leibniz Institute, Berlin, Germany,* [2]*Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russian Federation,* [3]*Charité - Universitätsmedizin Berlin, Medical Department I (Gastroenterology, Infectiology, and Rheumatology), Campus Benjamin Franklin, Berlin, Germany.*

In humans and mice, mucosal immune responses are dominated by IgA antibodies and the cytokine transforming growth factor beta (TGF-β), suppressing unwanted immune reactions but also targeting immunoglobulin class switching to IgA. IgA plays a central role in the interplay between the host cells and microbiota at the mucosal surfaces. The production of IgA as such is controlled by microbiota and requires the cytokine "transforming growth factor beta" (TGF-β), which induces IgA switch transcripts. However it has not been clear which bacteria direct production of IgA and how they do it. It had been suggested that eosinophils promote the generation and maintenance of mucosal IgA-expressing plasma cells. Here we demonstrate that not eosinophils, but specific bacteria enhance mucosal IgA production. We also now show that not the colonization of the intestinal tract and the stimulation of the intestinal immune with bacteria as such, but rather distinct microbial species lead to the preferential induction of intestinal IgA. Our data indicate that the bacteria of the genus *Anaeroplasma* increase numbers of IgA secreting plasma cells in the lamina propria of the small intestine, and significantly enhance mucosal IgA levels. *Anaeroplasma* controls IgA expression by inducing expression of the IgA class switch-inducing cytokine TGF-β in T follicular helper cells of Peyer´s patches. *Anaeroplasma* is also a part of the human microbiota. Its anti-inflammatory properties of inducing the immune regulatory cytokine TGF-β, strengthening the intestinal barrier by enhancing mucosal IgA, make it an interesting probiotic for the prevention and treatment of intestinal inflammation.

P.D1.04.02

Immune dysregulation in common variable immunodeficiency disorder: a role for *Enterococci*?

R. Berbers[1], *P. Ellerbroek*[1], *J. van Montfrans*[1], *M. Rogers*[1], *M. Viveen*[1], *F. Paganelli*[1], *V. Dalm*[2], *M. van Hagen*[1], *A. van de Ven*[3], *J. van Laar*[1], *R. Willems*[1], *H. Leavis*[1];
[1]*University Medical Center Utrecht, Utrecht, Netherlands,* [2]*Erasmus Medical Center, Rotterdam, Netherlands,* [3]*University Medical Center Groningen, Groningen, Netherlands.*

Common variable immunodeficiency disorder (CVID) is the most common primary immunodeficiency, but its etiology is not well understood. Currently, only 10% of cases can be explained by genetic variants. This humoral immunodeficiency is often accompanied by immune dysregulation phenomena, including autoimmunity, granulomas and lymphoproliferation. Better understanding of the cause of CVID and the immune dysregulation phenotype is key to improving care for these patients. Given the limited genetic contribution to CVID and prevalence of gastrointestinal symptoms in these patients, a role for the gut microbiome has been hypothesized.We aimed to characterise the composition of the fecal microbiota of 106 CVID patients and 49 healthy controls (HC) cross-sectionally. 16S rRNA profiling revealed decreased diversity in CVID patients compared to HC. Interestingly, IgA deficiency did not change alpha diversity within CVID patients. Although patients did not cluster separately from controls in unsupervised analyses, in unsupervised analyses distinct groups of bacteria were significantly associated with CVID. *Escherichia coli* was more abundant in CVID patients as compared to HC. *Enterococcus* spp were increased in patients with immune dysregulation (n=50) as compared to those without (n=56), regardless of use of medication. *Veillonella dispar* was associated with enteritis, while bacteria belonging to the *Clostridiaceae* and *Bifidobacteriaceae* families were more abundant in relatively healthier patients. To conclude, immune dysregulation in CVID was associated with low bacterial diversity and increased abundance of *Enterococci*, a group of bacteria that has recently been implicated in autoimmunity.

P.D1.04.03

Understanding the interaction between vaginal microbiota and immunity; the role of immunoglobulin A

***A. Breedveld**, H. Schuster, L. Pedró-Cos, R. Mebius, D. Budding, M. van Egmond;*
VUmc, Amsterdam, Netherlands.

Immunoglobulin (Ig) A is the most prevalent antibody at mucosal surfaces and alterations in IgA coating of gut-resident bacteria have been associated with inflammatory bowel disease. An unbalanced vaginal microbiota was recently linked to preterm birth, raising the question whether IgA is involved in this process. Still, much less is known about the interaction between IgA, bacteria and the immune system of the female genital tract.
We determined the vaginal microbial composition of healthy women using IS-pro. IgA coating of the vaginal microbiota from healthy donors was measured with flow cytometry. Additionally, the presence of specific IgA against common vaginal bacterial strains in serum was examined. Neutrophils, monocytes and monocyte-derived dendritic cells (moDCs) were stimulated with IgA-opsonized vaginal bacteria for 30min to measure phagocytic capacity. Cytokine production after 24h stimulation of cells with IgA-opsonized vaginal bacteria will be measured using ELISA.
Healthy vaginal microbial composition was predominated with *Lactobacillus spp*. Vaginal swabs contained a high proportion of IgA-coated bacteria. Neutrophils, monocytes and moDCs showed increased phagocytic capacity for IgA-opsonized vaginal bacteria. These results demonstrate a mucosal and systemic IgA response against vaginal bacteria. Cytokine profile of innate immune cells after stimulation with IgA-coated bacteria will be established and bacterial cell sorting as well as molecular microbiota analysis will be performed to study taxa-specific IgA coating. We aim to use IgA coating of vaginal bacteria as predictor for preterm birth.

P.D1.04.04

BCG vaccination in the childhood of women influences their placenobiomeduring pregnancy: vertical transmission of mycobacterial L forms

***T. Dimova**[1], A. Terzieva[1], L. Djerov[2], A. Nikolov[2], V. Dimitrova[2], P. Grozdanov[3], N. Markova[3];*
[1]Institute of Biology and Immunology of Reproduction "acad. K. Bratanov", Bulgarian Academy of Sciences, Sofia, Bulgaria, [2]Medical University, University Obstetrics and Gynecology Hospital "Mother House", Sofia, Bulgaria, [3]Institute of Microbiology "Acad. St. Angelov", Bulgarian Academy of Sciences, Sofia, Bulgaria.

Introduction: It has been shown that maternal microbiota drives early postnatal innate immune development and prepares the newborn for host-microbial mutualism by vertical transfer of microbial molecules. Recent studies with human placenta found the presence of dynamic placental microbiome with specific metabolic functions, which effects on the developing fetus and neonate is still unknown. Since live mycobacterial L-forms could be found in the blood of BCG-vaccinated people, we aimed to investigate a possible trans-placental transfer of BCG L-forms from the blood of healthy BCG-vaccinated pregnant women to their neonates as well as the presence of specific gamma/delta T-cell response in the placentobiome. Materials and methods: Sterile obtained samples from early pregnancy deciduas, term placentas, maternal blood and cord blood were examined by isolation and appropriate cultivation, electron microscopy, qPCR and FACS. Results: We proved that the majority of the samples of maternal blood, gestational tissues and cord blood of healthy newborns delivered by healthy BCG-vaccinated pregnant women were colonized with mycobacterial L-forms. The transfer of L-forms occurs early in gestation and the maternal decidua mediates the process of placenta colonization. No specific expansion of pathogen-reactive Vdelta2 gamma/delta T cells was detected. Conclusions: Novel data about mother-to-newborn transmission of mycobacterial L forms suggests that BCG vaccination in the childhood of the woman may affect her placentobiome during pregnancy.
Acknowledgments: This study was funded by Bulgarian National Science Fund, project DN 03/5.

P.D1.04.05

Effects of obesity on Tick-borne encephalitis (TBE) booster vaccination

E. Pöllabauer[*1], **E. Garner-Spitzer***[*1], A. Wagner[1], A. Guzek[1], I. Zwazl[1], C. Seidl-Friedrich[1], S. Schulz[1], C. Binder[2], K. Stiasny[3], M. Kundi[4], U. Wiedermann[1];*
[1]Medical University of Vienna, Institute of Specific Prophylaxis and Tropical Medicine, Vienna, Austria, [2]Dept. for Laboratory Medicine, Medical University Vienna, Austria, Vienna, Austria, [3]Center of Virology, Medical University Vienna, Austria, Vienna, Austria, [4]Center for Public Health, Medical University Vienna, Vienna, Austria.

Obesity has significantly increased worldwide and has, apart from related co-morbidities, direct effects on the immune system leading to immune dysfunction and increased susceptibility to infectious diseases. Thus prophylaxis against vaccine preventable diseases is particularly important in obese individuals. In order to assess vaccine efficacy, we performed an open-label phase IV clinical trial with 37 obese individuals and 36 normal-weight controls, which were booster-vaccinated against tick-borne encephalitis (TBE). The general immunologic and metabolic profile along with vaccine-specific humoral and cellular immune responses were evaluated in sera and PBMC. Obese adults showed significantly increased metabolic (leptin, insulin, triglycerides, cholesterol) and pro-inflammatory (CRP) markers. Total immunoglobulin levels (IgM, IgA) were increased in obese vaccinees, while natural IgM against pathogens (PC) were significantly reduced, possibly indicating higher infection susceptibility. Obese individuals showed a stronger fold-increase of TBE-specific Ab titers 4 weeks after booster, which was positively correlated with metabolic parameters. However, Ab levels declined significantly faster within 6 months in this group. At cellular level, TBE-specific cytokines (IL-2, IFN-γ) were increased in obese vaccinees before vaccination and distributions of B- and T-cell subsets differed between obese and control group. Vaccine reactogenicity (local and systemic) was higher in obese subjects. Our results indicate that TBE booster vaccination was effective at humoral and cellular level in obese individuals; however, the higher decline rate in obese might lead to shorter long-term protection. Whether the effects of obesity on primary TBE vaccination are similar remains to be investigated. *contributed equally Supported by investigator initiated industrial funding (Pfizer)

P.D1.04.06

Study on the effect of metformin and succinate on the differentiation and functions of mesenchymal stem cell

***H. Ho**[1], B. Chiang[2];*
[1]Graduate Institute of Oral Biology, School of Dentistry, Taipei, Taiwan, [2]Graduate Institute of Clinical Medicine College of Medicine of National Taiwan University, Taipei, Taiwan.

Introduction: Mesenchymal stem cells (MSCs) have been used for a variety of diseases due to their unique properties. Metabolic regulation including metabolic modulators treatment and glucose supplementation has indicated to enhance MSCs properties. But the metabolic regulation to immunomodulatory function of MSCs remains unclear. Hyperglycemia induces inflammation and impairs TCA cycle, causing immunity-related succinate accumulated in T2D, which can treat by MSCs and metformin. Combined metformin and MSCs could be a possible choice for T2D. Hence, we investigated the immunomodulation of metformin on the MSCs, including clarifying the role of succinate in the mechanisms.
Materials and Methods: MSCs are derived from the bone marrow of the 4-5 week old female BALB/c mice. Isolated-MSCs are treated by metformin or succinate to assay the immunosuppressive function and differentiation capacity. The gene expression of immunoregulatory factors and metabolic change are detected by Q-PCR.
Results: we found that metformin and succinate could enhance suppressive function to T cell and also the differentiation capacity. The mRNA level of iNOS increases in treated-MSCs but decreased after treating with INF-γ, which is required for MSC-mediated immunosuppression activation. Treated-MSCs change the metabolic genes including glycolysis, oxidative metabolism and mitochondrial biogenesis. The supernatant of treated-MSCs also demonstrated enhanced suppressive function, indicating the potential mechanism of treated-MSCs.
Conclusions: Metformin- or succinate-treated MSCs demonstrated enhanced suppressive function and different metabolism. We believe further clarification on the metabolic mechanisms involved in the development and functions of MSCs might shed light on future application of MSCs for immune modulation.

P.D1.04.07

Metronidazol changes the susceptibility to imiquimod-induced skin inflammation

***Z. Jiraskova Zakostelska**[1], Z. Stehlikova[1], F. Rob[2], K. Klimesova[1], P. Rossmann[1], I. Novosadova[1], D. Srutkova[3], M. Kostovcik[1,4], T. Hudcovic[3], P. Bohač[2], K. Juzlova[2], J. Hercogova[2], H. Tlaskalova Hogenova[1], M. Kverka[1,5];*
[1]Institute of Microbiology of the CAS, Prague, Czech Republic, [2]2nd Medical Faculty, Charles University in Prague and Bulovka Hospital, Prague, Czech Republic, [3]Institute of Microbiology of the CAS, Novy Hradek, Czech Republic, [4]BIOCEV, MBU-BIOCEV, Vestec, Czech Republic, [5]Institute of Experimental Medicine of the CAS, Prague, Czech Republic.

Introduction: Alteration of microbiota influences the immune response of the host. Overall changes in microbiota composition are also involved in the psoriasis incidence and maintenance. The aim was to reveal whether peroral antibiotic treatment has the potential to mitigate experimental skin inflammation and to describe in detail subsequent changes in microbiota composition on the skin and in the intestine. **Methods:** To alter the microbiota, we gavaged BALB/c mice with antibiotics, specifically metronidazole (MET), vancomycin, colistin and streptomycin, and their mixture (ATBs) or water (W) in controls consecutively for 21 days. To induce the imiquimod-induced skin inflammation (IISI), we applied imiquimod on the shaved back skin for last 6 days. Then, we evaluated the severity of IISI. We used Illumina approach to assess the changes in microbiota. **Results:** Compared to W control mice, we found that MET and ATBs mitigated the severity of IISI in all clinical parameters. This effect was associated with downregulation of Th17 response. Next, we found that treatment with MET and ATBs significantly decreased the intestinal microbial diversity but microbial diversity on skin was significantly changed only after treatment with MET. Although, ATBs increased the proportion of phylum Firmicutes in the intestine, particularly order *Lactobacilales*, other members, such as *Clostridiales* and *Bacillales*, were decreased. **Conclusions:** Our findings suggest that differences in microbiota composition can influence experimental skin inflammation development and can lead to identification of specific microbial patterns associated with the disease. This study was supported by Ministry of Health of the Czech Republic grant nr. 15-30782A

P.D1.04.08

Impact of Foxp3$^+$ Regulatory T cell depletion on the murine gut microbiome

C. Wilk, D. Schoemer, A. Adamczyk, E. Pastille, A. Westendorf, J. Buer, ***J. Kehrmann****;*
Institute of Medical Microbiology, Essen, Germany.

Introduction: Besides the impact of the gut microbiota to shape the immune system in the intestine, the immune system itself is considered to shape the gut microbiota composition. The relevance of regulatory T cells (Tregs) in this context is unclear so far. We analyzed the gut microbiome in DEREG mice over a period of twenty days after depletion of Tregs by Diphtheria Toxin (DT).
Methods: We extracted DNA from stool samples of twenty-four mice (19 DEREG and 5 wild-type mice) at 5 different time points (day -7 [seven days before first DT application], day 0, 5, 10 and 20), derived from 3 independent experiments. Sequencing of the V3/V4 region of the 16S rRNA gene was performed using the Illumina MiSeq 2 x 301 paired ends reads cartridge (Illumina). Data processing and analysis was performed using the QIIME pipeline.
Results: Variation of the gut microbiome´s alpha and beta diversity was clearly higher between individual mice compared to the different time points of stool sampling within mice of this study. Principle Coordinate Analysis (PCoA) of weighted and unweighted UniFrac distance matrices did not separate DEREG and wildtype mice gut microbiomes. PCoA clustered samples according to their sample identifier and age rather than according to the different time points before and after Treg depletion.
Conclusions: In this work we analysed the gut microbiome variation associated with Treg depletion in mice over time. Intra-mice variability of the gut microbiome over time was less pronounced compared to inter-mice variability before Treg depletion.

P.D1.04.09

Expression of Toll-like receptors in PBMCs of schizophrenic patients

E. Kozlowska*[1], J. Agier[1], K. Sobierajska[2], E. Brzezińska-Blaszczyk[1], A. Wysokinski[3];*
[1]Department of Experimental Immunology, Medical University of Lodz, Lodz, Poland, [2]Department of Molecular Cell Mechanisms, Medical University of Lodz, Lodz, Poland, [3]Department of Old Age Psychiatry and Psychotic Disorders, Lodz, Poland.

Background. Schizophrenia is a severe mental disorder characterized by firmly impaired thinking, emotions, and behaviors, affecting approximately 1% of the population worldwide. Increasing evidence suggests that, apart from neurochemical abnormalities, various immunological alterations are related to the pathogenesis of schizophrenia. Given a fact that Toll-like receptors (TLRs) play pivotal role in the initiation of innate immunity and inflammatory mechanisms in this study we determine the TLR expression in peripheral blood mononuclear cells (PBMCs) in schizophrenic patients.
Materials and methods. Twenty-seven adult European Caucasian patients with paranoid schizophrenia were included in this prospective study. Twenty-nine healthy volunteers were also randomly selected as a control group. PBMCs were isolated from whole blood by density gradient centrifugation. Total RNA was isolated from PBMCs by acid guanidinium thiocyanate-phenol-chloroform extraction. TLRs expression was evaluated using quantitative real-time polymerase chain reaction (qRT-PCR).
Results. We demonstrated that TLR1, TLR2, TLR4, TLR6, and TLR9 mRNAs expression were down-regulated in patients with schizophrenia in opposite to TLR3 and TLR7 mRNAs which manifested higher expression. TLR5 and TLR8 mRNAs demonstrated non-statistically significant alterations.
Conclusion. Altered expression of TLRs in PBMCs of schizophrenic patients may reflect an important interplay between TLRs and development of schizophrenia.
Supported by the Medical University of Lodz (grant no 502-03/6-164-01/502-64-106).

P.D1.04.10

Roles of IRF-1 in negative cross-talk between Liver X Receptors (LXRs) and IFN-γ signalling

N. A. Letelier*[1], L. Apetoh[2], A. M. Planas[3], A. F. Valledor[1];*
[1]Nuclear Receptor Group, Department of Cell Biology, Physiology and Immunology, School of Biology, University of Barcelona, Barcelona, Spain, [2]Centre de Recherche INSERM LNC-UMR1231, F-21000, Dijon, France, [3]Department of Brain Ischemia and Neurodegeneration, Institute for Biomedical Research of Barcelona (IIBB), Consejo Superior de Investigaciones Clentíficas (CSIC), Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.

Liver X Receptors (LXRs) are members of the nuclear receptor family of transcription factors that regulate lipid and glucose homeostasis and exert several roles at the interface between metabolism and the immune system. Pharmacological LXR activation induces the expression of genes involved in cholesterol efflux (e.g. *Abca1* and *Abcg1*) and inhibition of cholesterol uptake (*Idol*). Recent work from our group has revealed that LXR activation also induces the expression of the enzyme CD38 in macrophages, a transmembrane glycoprotein that controls NAD+ levels in tissues. Further, we have previously reported reciprocal cross-talk between IFN-γ signalling and the LXR pathway in macrophages. IFN-γ involves activation of the JAK-STAT1 pathway to promote the expression of primary IFN response genes, including the transcriptional regulator IFN regulatory factor-1 (IRF-1). To characterize the roles of IRF-1 in the cross-talk between IFN-γ and LXR activation, we compared the effects of IFN-γ stimulation on the expression of LXR target genes in macrophages from wild type and IRF-1 or STAT1-deficient mice. IFN-γ signalling resulted in reduced induction of *Abca1* and *Aim* by LXR, effects that were STAT1-dependent but IRF-1-independent. In contrast, IFN-γ signalling and LXR cooperated in transcriptional induction of *Cd38* in a STAT1-dependent manner and this synergistic induction was drastically enhanced in IRF-1-deficient macrophages. These results suggest that IRF-1 serves to fine-tune the expression of CD38 during the macrophage response to IFN-γ.
This work was supported by grant 201605.31 from Fundació La Marató de TV3 to A.F.Valledor; Nicole Letelier is a CONICYT fellowship awarded.

P.D1.04.11

Short-chain fatty acids regulate systemic bone mass and protect from pathological bone loss

S. Lucas*[1], Y. Omata[1], J. Hofmann[2], M. Böttcher[3], A. Iljazovic[4], K. Sarter[1], O. Albrecht[1], O. Schulz[1], B. Krishnacoumar[1], G. Krönke[1], M. Herrmann[1], D. Mougiakakos[3], T. Strowig[4], G. Schett[1], M. M. Zaiss[1];*
[1]Department of Internal Medicine 3, Rheumatology and Immunology, Friedrich-Alexander-University Erlangen-Nürnberg (FAU) and Universitätsklinikum Erlangen, Erlangen, Germany, [2]Division of Biochemistry, Department of Biology, Friedrich-Alexander University Erlangen-Nürnberg (FAU), Erlangen, Germany, [3]Department of Internal Medicine 5, Hematology and Oncology, Translational Research Center, University Hospital Erlangen, Erlangen, Germany, [4]Helmholtz Centre for Infection Research, Braunschweig, Germany.

Host-microbe interactions are considered being a fundamental component influencing health and disease. Especially the gut microbiota and their metabolites are known to modulate immune responses of the host. The main metabolites derived from microbial fermentation of dietary fibers are short chain fatty acids (SCFA). SCFA have been recognized as potential mediators involved in the effects of the gut microbiota on local and systemic immune function.
Because immune activation is intimately linked to bone homeostasis, we analyzed the effect of SCFA on bone.
Therefore, we characterized different mouse models addressing physiological bone homeostasis in naïve C57BL/6 and Rag1$^{-/-}$ mice as well as models for post-menopausal and inflammation driven pathological bone loss during SCFA supplementation or fiber rich diets.
The analysis of tibial bone revealed that SCFA increased bone mass during physiological conditions by directly impacting on the metabolic status of osteoclast precursors, shifting it at early time points towards enhanced glycolysis. Moreover, propionate and butyrate attenuated ovariectomy-induced bone loss as well as the severity of inflammation during collagen induced arthritis, and subsequently preserved local and systemic bone mass.
SCFA appear to be potent regulators of bone homeostasis, and are also able to prevent pathological bone loss. These data suggest that microbial homeostasis in the gut associated with adequate production of SCFA is an important regulatory element in determining bone composition in mice. Therapeutic supplementation of SCFA or diets increasing the endogenous production of SCFA may therefore provide a powerful instrument to balance osteoclast activity and prevent enhanced bone resorption.

P.D1.04.12

Gut microbiota-derived metabolite pentanoate restrains T cell-mediated immunopathology through metabolic and epigenetic reprogramming of B and T lymphocytes

M. Luu*[1], R. Romero[1], R. Singh[2], M. Lauth[2], U. Steinhoff[1], A. Visekruna[1];*
[1]Institute for Medical Microbiology and Hygiene, Philipps-University Marburg, Marburg, Germany, [2]Institute of Molecular Biology and Tumor Research, Philipps-University Marburg, Marburg, Germany.

Short-chain fatty acids (SCFAs) are able to induce differentiation of regulatory T cells (Tregs), which is one of the crucial mechanisms by which commensal bacteria contribute to the maintenance of mucosal homeostasis. SCFAs are able to exert protective effects in the host cells through both G-protein-coupled receptor (GPCR)-dependent and -independent mechanisms. While a potent immunomodulatory activity for acetate, propionate and butyrate has been described in various experimental models, the role for the SCFA pentanoate (valerate) in regulation of immune cell function is still unknown. Here, we show that pentanoate is abundantly present in the gut lumen of conventional but not of germ-free (GF) mice. Interestingly, pentanoate was not able to expand regulatory T cells (Tregs) *in vitro* or *in vivo*. In CD4$^+$ T lymphocytes, pentanoate acted as epigenetic regulator of gene expression by exhibiting strong histone deacetylase (HDAC)-inhibitory activity, thereby reducing IL-17A production and suppressing pathogenic Th17 responses *in vivo*. Furthermore, pentanoate strongly enhanced IL-10 production in regulatory B cells (Bregs) and Th17 cells by reprogramming their metabolic activity. This effect was dependent on pentanoate-triggered metabolic switch towards high aerobic glycolysis via enhancement of mTOR activity. Pentanoate-induced IL-10$^+$ Bregs mediated protection in the mouse model of colitis and multiple sclerosis. Taken together, our study reveals that the microbiota-derived metabolite pentanoate regulates the fate of lymphocytes by initiating metabolic reprogramming in these cells.

P.D1.04.13

Microbiome composition of air samples from livestock farms and their effect on innate immune receptors and cells

***R. Mariman**, D. Liu, M. Gerlofs-Nijland, B. John, F. Cassee, E. Pinelli;*
RIVM, Bilthoven, Netherlands.

Patients with respiratory diseases in rural areas have been reported to have enhanced responsiveness to ambient particulate matter (PM). In addition to the physical and chemical components, ambient PM can contain microorganisms or parts thereof which is referred here as BioPM. The aim of this study is to characterize the microbial composition of BioPM originating from livestock, and to investigate whether these BioPM can trigger the activation of innate receptors and cells. Size-resolved BioPM samples (<2.5 and 2.5-10 micrometer) were collected from chicken, pig and goat farms using the versatile aerosol concentration enrichment system (VACES) connected to a Biosampler. The fungal and bacterial community was assessed with an amplicon based approach using Next Generation Sequencing. In parallel, HEK-Blue cells expressing different pattern recognition receptors (Toll like receptors (TLR) 2,3,4,5,7,8,9 and NOD1,2) and a human monocytic cell line (MM6) were exposed to BioPM from these sites. Results indicate distinct airborne microbiota profiles associated with the corresponding animal farm. Moreover, the various BioPM contained mainly ligands for TLR2 and TLR4 resulting in a concentration-dependent increase of pro-inflammatory cytokines secreted by MM6 cells. In addition to TLR2 and TLR4 only the pig-derived BioPM induced TLR5 activation. These findings indicate that BioPM from livestock can activate innate cells and therefore, trigger inflammatory responses. Knowledge on the microbial composition of BioPM derived from different farms and understanding what type of inflammatory responses they induce is crucial for future studies on the effect of that exposure to BioPM may have on respiratory symptoms.

P.D1.04.14

Lactobacilli secreted factors dampen human IFN-γ responses through a monocyte-dependent mechanism

***M. Mata Forsberg**, S. Björkander, E. Sverremark-Ekström;*
The Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm, Sweden.

Lactobacilli are common members of the human microbiome known for their immunomodulatory function. We have previously shown that lactobacilli cell free supernatants (CFS) dampen T cell and NK cell pro-inflammatory cytokine production, proliferation and cytotoxicity, *in vitro*. However, the mechanisms involved remain elusive. Here, we aimed to investigate how soluble factors present in the lactobacilli-CFS mediate the dampening activity. We stimulated whole human PBMC or PBMC depleted of antigen presenting cells in the presence or absence of size fractionated lactobacilli-CFS. Furthermore, conditioned cell culture media from isolated monocytes pre-treated with lactobacilli-CFS was used in subsequent PBMC stimulations. Cytokine production was analyzed with ELISA and flow cytometry. We show that lactobacilli-CFS contains multiple soluble factors of different molecular size capable of reducing IFN-γ expression in NK cells and several T cell subsets. Moreover, the dampening activity is lost when monocytes, but not B cells, are depleted from the PBMC cultures prior to stimulation. Conditioned cell culture media from isolated monocytes pre-treated with lactobacilli-CFS also resulted in reduced IFN-γ secretion from PBMC. In summary, we show that lactobacilli-CFS dampen pro-inflammatory immune responses via a mechanism that involves soluble factors produced by monocytes.

P.D1.04.15

Metabolic reprogramming towards aerobic glycolysis might control the antigen presentation capacity of CD4+ T cells

***J. Osuna Pérez**, R. García-Ferreras, A. Cruz-Adalia, M. Torres Torresano, E. Veiga Chacón;*
Spanish National Center for Biotechnology, 28049, Spain.

Conventional $CD4^+$ T cells have recently been identified as potent inductors of cytotoxic memory $CD8^+$ T cells responses. By a process termed transphagocytosis T $CD4^+$ T cells capture bacteria from infected dendritic cells and degrade it to present bacterial antigens to $CD8^+$ T naive T cells, which proliferate and become cytotoxic in responses. This T $CD4^+$ T cell-mediated antigen presentation generates central memory T $CD8^+$ cells with low PD-1 expression and provide anti tumor protection, highlighting the potential of $CD4^+$ T cells a tool for cancer immunotherapy. Among the complex field of immunometabolism, the present study aims to determine the association between the metabolic state of transphagocytic $CD4^+$ T cells and their ability to present bacterial antigens to $CD8^+$ T cells. A higher rate of glycolysis was found in tp$CD4^+$ T cells when compared with activated $CD4^+$ T cells and disruption of the glycolytic pathway with 2-Deoxyglucose impaired T $CD8^+$ cells proliferation induced by $CD4^+$. Thus, we provide mechanistic insights into the metabolic reprogramming that gover the generation of central memory and antitumor response induced by T $CD4^+$ cells.

P.D1.04.16

X-linked Chronic Granulomatosis: molecular and cellular mechanisms underlying intestinal inflammation

***M. Pellicciotta**[1], M. Chiriaco[2], S. Di Cesare[3], E. Fontana[1], S. Guglielmetti[4], R. Rigoni[1], F. Rea[5], V. Marrella[6], A. Finocchi[3], B. Cassani[7];*
[1]Humanitas Clinical and Research Hospital, ROZZANO (MILANO), Italy, [2]University Department of Pediatrics, Childrens' Hospital Bambino Gesù, Rome, Italy, roma, Italy, [3]University Department of Pediatrics, Childrens' Hospital Bambino Gesù, Rome, Italy, Roma, Italy, [4]Department of Food, Environmental, and Nutritional Sciences (DeFENS), University of Milan, Italy, MILANO, Italy, [5]Digestive Endoscopy and Surgery Unit, Bambino Gesù Children Hospital, Roma, Italy, [6]Istituto di Ricerca Genetica e Biomedica, Milan Unit, CNR, Milan, Italy, MILANO, Italy, [7]Istituto di Ricerca Genetica e Biomedica, Milan Unit, CNR, Milan, Italy, ROZZANO (MILANO), Italy.

X-linked Chronic Granulomatous Disease is immunodeficiency disorder of phagocytes, due to defect in the CYBB gene, encoding the gp91phox subunit of the NADPH enzyme, resulting in impaired killing of bacteria and fungi. The enzyme is expressed also in lymphocytes but its functional implication is poorly characterized. Affected individuals develop life-threatening infections and unexplained autoinflammatory/autoimmune conditions, involving particularly the intestine. Increased tissue expression of pro-inflammatory cytokines (IL-17, IL-6, IL-1beta) underlined the intestinal inflammation in the gp91phox-/- mice. Treg cell frequency in the mesenteric lymph nodes was reduced in mutants as well as the percentage of CD103+ dendritic cells, endowed with tolerogenic functions. Consistently, OVA-specific Treg conversion, analyzed in vivo upon adoptive transfer of CD4+ OTII cells and oral antigen delivery, was affected in gp91phox-/- mice. Interestingly, IgA compartment, crucial to contain gut microbes, was similarly defective in the knock-out mice. PBMC of 13 CGD patients and of 10 age-matched HD were evaluated by flow cytometry. Eight out of 13 patients suffered from IBD with predominant colonic involvement. Analysis at the enrollment showed diminished naïve CD4 and CD8 subsets and increased effector memory (CD45RA-CD27- and CD45RA-CCR7-) cells as well as a slight increase in the NKT subset. Despite normal B cell frequencies, the memory subsets (CD19+CD27+ unswitched and switched memory CD19+CD27+IgD-), were all below the normal range values. Studies are underway to investigate the presence of rare genetic variants associated with IBD. Furthermore, gene and protein expression from intestinal biopsies will be correlated with microbiota composition and fecal IgA content.

P.D1.04.17

The influence of sex chromosome-specific gut microbiomes on a sexually dimorphic immune response

*A. Pettit[1], J. Franko[1], E. Onga[2], K. Blethen[1], C. Cuff[1], **R. Schafer**[1];*
[1]West Virginia University, Morgantown, United States, [2]Universidade Federal De Vicosa, Vicosa, Brazil.

Despite being less susceptible to infectious disease, females are 10X more likely to develop autoimmune diseases than males. Immune-related sexual dimorphisms have primarily been attributed to sex hormones, however, XX vs. XY sex chromosome complements and sex-specific gut microbiomes may play a role. To identify mechanisms contributing to distinct male vs. female immune responses, the gut microbiome composition of four-core genotype (4CG) mice and its influence on a sexually dimorphic immune response was evaluated. FCG mice exhibit one of 4 genotypes: XX or XY females (ovaries) and XX or XY males (testes). 4CG mice have been used to identify differences in phenotypes caused by sex chromosome complements, sex hormones, and interactions between the two. 16s rDNA sequencing and metagenomics analysis was performed on DNA isolated from the fecal pellets of 6-8 week old 4CG mice and again 4 weeks post-gonadectomy. Distinct populations of bacteria were identified between XX females and XY males, and also between XX and XY males, suggesting a role for sex chromosome complements in the regulation of gut microbiome composition. Elimination of gut bacteria by antibiotic administration demonstrated a potential role for sex chromosome-specific gut microbiomes on the XX-dependent enhancement of heat-killed *Streptococcus pneumoniae* immune responses after exposure to the herbicide, propanil. Future experiments will determine the metabolic by-products produced by the microbiome of 4CG mice and determine if these metabolites influence immune responsiveness in a sex chromosome-dependent manner. Funded by the Department of Microbiology, Immunology, and Cell Biology Research Internship and NIH Grant P20GM103434, WVINBRE.

P.D1.04.18

Psoriasis related changes in skin microbiota composition

***Z. Stehlikova**[1], K. Juzlova[2], F. Rob[2], J. Hercogova[2], A. Uzan[3], O. Koren[3], M. Kostovčik[1,4], H. Tlaskalova Hogenova[1], Z. Jiraskova Zakostelska[1];*
[1]Institute of Microbiology of the CAS, v.v.i., Prague, Czech Republic, [2]Bulovka Hospital, 2nd Faculty of Medicine, Prague, Czech Republic, [3]Bar-Ilan University, The Azrieli Faculty of Medicine, Safed, Israel, [4]BIOCEV, Institute of Microbiology of the CAS, v.v.i., Vestec, Czech Republic.

Introduction: Psoriasis is an immune mediated, chronic, inflammatory skin disease of unknown etiology affecting 2-3% of the worldwide population. It is widely accepted that it develops from a combination of genetic and environmental triggers, such as stress, bacterial infection, antibiotics, and diet. However, the role of microbiota in its pathogenesis remains still unclear. The aim of this study was to describe microbiota composition in psoriatic patients and compare it to healthy controls.
Methods: We collected samples from the human back skin using two sampling techniques: swabbing, and scraping. Subsequently, we compared microbiota composition of affected psoriatic skin with unaffected contralateral skin from the same patient and with healthy controls after sequencing V1V2 region of 16S rRNA on Illumina MiSeq platform. Data were analyzed using QIIME.

Results: Intergroup β-diversity showed significantly higher microbiome variability among controls in contrast to higher similarity between psoriatic patients including both affected and unaffected skin. Intergroup α-diversity revealed increasing bacterial diversity from controls to affected psoriatic lesions, with a decrease of *Staphylococcus epidermidis*, increase of *Corynebacterium* species and appearance of pathogenic *Staphylococcus haemolyticus* in psoriatic patients. Moreover, we observed proportional changes in the bacterial distribution in between the sampling techniques used.
Conclusions: Our results confirmed that psoriasis is associated with changes in skin microbiota. However, whether it is of its primary etiological significance or secondary to the disease remains to be established.
This study was supported by Ministry of Health of the Czech Republic, grant No.15-30782A, RVO: 61388971 and by European Regional Development Fund BIOCEV CZ.1.05/1.1.00/02.0109.

P.D1.04.19
Human gut microbes are highly susceptible to antimicrobial food additives *in vitro*

L. Hrncirova[1,2]*, E. Sukova*[1]*, T. Hudcovic*[1]*, J. Krejsek*[2]*, T. Hrncir*[1]*;*
[1]*Institute of Microbiology, Czech Academy of Sciences, Novy Hradek, Czech Republic,* [2]*Faculty of Medicine in Hradec Kralove, Charles University in Prague, Hradec Kralove, Czech Republic.*

The aim of this project was to test the hypothesis that antimicrobial food additives may alter the composition of human gut microbiota by selectively suppressing the growth of susceptible gut microbes. To explore the influence of antimicrobial food additives on the composition of the human gut microbiota, we examined the susceptibility of both aerobic and anaerobic gut bacteria to sodium benzoate, sodium nitrite, and potassium sorbate, and their combinations, using a broth microdilution method. The tested bacteria showed a range of susceptibilities to the different food additives, with *Bacteroides coprocola* and *Clostridium tyrobutyricum* being particularly sensitive. However, most importantly, we found that gut microbes with known anti-inflammatory properties were mostly susceptible to the antimicrobial food additives, while microbes with known pro-inflammatory or colitogenic properties were mostly resistant. Our data show that some human gut microbes are highly susceptible to antimicrobial food additives. We speculate that permanent exposure of human gut microbiota to even low levels of additives may modify the composition and function of gut microbiota and thus influence the host's immune system. Whether the effect of additive-modified gut microbiota on the human immune system could explain, at least in part, the increasing incidence of allergies and autoimmune diseases remains to be shown.

P.D1.04.20
Food preservatives induce *Proteobacteria* dysbiosis of the human gut microbiota

T. Hrncir[1]*, L. Hrncirova*[1,2]*, E. Trckova*[1]*, V. Machova*[1]*, J. Krejsek*[2]*;*
[1]*Institute of Microbiology, Czech Academy of Sciences, Novy Hradek, Czech Republic,* [2]*Faculty of Medicine in Hradec Kralove, Charles University in Prague, Hradec Kralove, Czech Republic.*

The incidence of allergies and autoimmune diseases is increasing worldwide. Recent data suggest that gut microbiota can modulate not only local but also systemic immune responses. In this study, we focus on environmental factors, specifically food preservatives, which may modify the composition of gut microbiota and thus influence host's immune responses. To address this issue, we have administered either sterile water or water supplemented with additives to wild-type and Nod2-deficient C57BL/6 mice colonized with human microbiota. The daily intake of additives was calculated to match the maximum daily intake reached in human populations in Europe. We have analyzed the effect of additives on microbial composition and diversity by amplification and high-throughput sequencing of the hypervariable regions of the 16S rDNA genes. The resulting sequences were processed using QIIME2 software package. Our results indicate that commonly used food preservatives can decrease the diversity of the human gut microbiota and also trigger *Proteobacteria* dysbiosis.

P.D1.04.21
European XFEL, The world's largest and powerful X-ray free-electron laser

D. MEZA*, J. Guel, R. Schubert, E. Round, H. Han, J. Makroczcyova, K. Lorenzen, J. Schulz;*
EUROPEAN XFEL, SCHENEFELD, Germany.

The European XFEL generates ultrashort X-ray flashes 27 000 times per second and with a brilliance that is a billion times higher than that of the best conventional X-ray radiation sources**. How it works.** To generate the X-ray flashes, bunches of electrons are first accelerated to high energies and then directed through special arrangements of magnets. Electrons are first brought to high energies in a superconducting accelerator. They then fly on a slalom course through a special arrangement of magnets in which they emit laser like flashes of radiation. **Instruments**. scientists can make use of sophisticated instruments to carry out their experiments such as SPB/SFX (ultrafast coherent diffraction of single particles: structure determination of single particles like atomic clusters, biomolecules, virus particles, cells and serial femtosecond crystallography, the FXE scientific instrument serve a broad scientific community and embrace several fields of ultrafast X ray science and their applications. Delivering new information serving applications in many fields, the XFEL Biology Infrastructure facility with all its equipment provide versatile tools to significantly increase the feasibility of a wide variety of experiments on biological samples. **Scope**. The X-ray flashes are so short that scientists are able to use them to film ultrafast phenomena such as the formation or breakup of chemical bonds and also make possible to research the composition and structure of complex biomolecules such as proteins, cells, or membranes on the atomic scale providing insights into their functions, offering important basis for the development of future medicines and therapies.

P.D2.01 Innate Lymphoid Cells

P.D2.01.01
A cell-autonomous role of DOCK8 in development of type3 innate lymphoid cells

R. Aihara[1]*, M. Watanabe*[1]*, Y. Fukui*[1,2]*;*
[1]*Medical Institute of Bioregulation. Kyushu University, Fukuoka, Japan,* [2]*Reseach Center for Advanced Immunology, Kyushu University, Fukuoka, Japan.*

Introduction: RORgt-expressing ILC3s play an important role in the defense against intestinal pathogens and promotion of epithelial homeostasis via production of IL-22. However, the mechanism controlling ILC3 development is not fully understood. In this study, we examined the role of DOCK8, an atypical guanine nucleotide exchange factor for Cdc42, in ILC3 development. Material and methods: Conventional DOCK8 KO mice and control mice were used for FACS analyses and immunohistochemical analyses to examine ILC3 development and intestinal fucosylation. The conditional KO mice lacking DOCK8 in RORgt-positive cell lineage were developed and used to examine its effect on ILC3 development. Results: DOCK8 KO mice exhibited a severe reduction of RORgt+ c-Kit+ Sca1- ILC3s in intestinal lamina propria. Consistent with this, lineage-negative cells producing IL-22 were also reduced in the KO mice under the both steady state and IL-23-treated condition. IL-22 is known to mediate the epithelial fucosylation. Although the epithelial fucosylation was severely impaired in the KO mice, IL-22 administration completely restored the fucosylation response. To examine whether DOCK8 regulates ILC3 development in a cell-autonomous manner, we generated the conditional DOCK8 KO mice. When the conditional KO mice were crossed with RORgt-Cre mice, ILC3 development was impaired, as was observed in conventional KO mice. In contrast, genetic deletion of DOCK8 in CD11c-positive cell lineage did not affect ILC3 development. Conclusion: DOCK8 regulates ILC3 development in a cell-autonomous manner.

P.D2.01.02
Enrichment of innate lymphoid cell populations in murine gingival tissue

J. L. Brown[1,2]*, L. Campbell*[2]*, J. Malcolm*[2]*, A. Adrados Planell*[2]*, J. Butcher*[1,2,3]*, S. Culshaw*[2]*;*
[1]*University of West of Scotland, Paisley, United Kingdom,* [2]*University of Glasgow, Glasgow, United Kingdom,* [3]*Glasgow Caledonian University, Glasgow, United Kingdom.*

Introduction: Innate lymphoid cells (ILCs) are lymphocytes that act as the first line of immunological defence at mucosal surfaces such as the gut, lungs and the skin. Here, we provide a detailed appraisal of the whole ILC population (group 1, 2 and 3 subsets) in the murine gingivae and the regional lymph nodes (dLNs) draining the oral cavity.
Methods: Oral dLNs and gingivae were harvested from mice and processed/digested to obtain single cell suspensions, which were subsequently stained with antibodies for identification of ILCs by flow cytometry. For cytokine profiling of ILCs, cells were stimulated prior to staining.
Results: We show that ILCs made up a greater percentage of the whole CD45+ lymphocyte population in the murine gingivae than in the oral dLNs (0.356 ± 0.039% vs. 0.158 ± 0.005%, $p<0.001$). The gingivae-resident ILCs were more diverse than the oral dLNs, with a significantly greater proportion of CD117+, NKp46- ILCs (35.19 ± 3.84 compared to 5.03 ± 0.69%, $p<0.0001$). The cytokine profile of ILCs in the gingivae also differed from the oral dLNs; there was a relatively similar proportion of IFN-γ+ and IL-5+ ILCs in the murine gingivae, whereas IL-5+ ILCs predominately populated the oral dLNs.
Conclusion: The function of ILCs in the oral cavity is currently unknown; here, we demonstrate that the ILC compartment is enriched, more diverse, and has a different cytokine profile at the gingival surface compared to the oral dLNs. Future work investigating inflammatory oral diseases using mouse models may merit consideration of these ILC populations.

P.D2.01.03
CD62L marks circulating ILC precursors

*Y. E. Bar-Ephraim, **E. Burniol Ruiz**, J. Koning, T. Konijn, V. P. Mourits, K. Lakeman, L. Boon, M. Bögels, J. van Maanen, J. J. Den Haan, M. Van Egmond, G. Bouma, R. M. Reijmers, R. Mebius;*
VUmc, Amsterdam, Netherlands.

Innate lymphoid cells (ILCs) guard epithelial tissue integrity during homeostasis, but can be potent immune effector cells during inflammation. Precursors to all ILC subsets (ILC precursors, ILCP) have been identified in human peripheral blood (PB). We found that during homeostasis, ILCP in PB of mouse and man expressed homing receptors for secondary lymphoid organs, mainly CD62L. These ILCP entered mouse lymph nodes (LNs) in a CD62L-dependent way and relied on S1P receptors for their exit. Importantly, CD62L expression was absent on human ILCs expressing NKp44 in tonsils and PB of Crohn's disease (CD) patients, and reduced number of CD62L$^+$ ILCP were present in PB of CD patients. These data are in agreement with selective expression of CD62L on non-activated ILCP. As such, we conclude that CD62L not only serves as a functional marker of ILCP but has potential to be used in the clinic as a diagnostic marker in inflammatory disorders.

P.D2.01.04
Dynamics of innate lymphoid cells (ILCs) during *Mycobacterium tuberculosis*infection

***D. Corral**, F. Levillain, O. Neyrolles, D. Hudrisier;*
Institut de Pharmacologie et de Biologie Structurale, Toulouse, France.

Understanding the immune response to *Mycobacterium tuberculosis* (*Mtb*), the etiological agent of tuberculosis (TB) may help propose innovative therapeutic approaches. In this context, the role of innate lymphoid cells (ILCs), which form a recently identified group of innate lymphocytes, remains unexplored. ILCs are preferentially located at mucosal surfaces where they contribute to controlling immunity. The aim of our project is to analyze the dynamics of ILCs upon *Mtb* infection in the murine model and to understand their contribution to the balance between immune-driven protection and pathology, key parameters in ensuring *Mtb* elimination while preserving functional integrity of the lung tissue during TB. Our data show that, during *Mtb* infection, ILC subsets are differentially recruited to the lungs where they are activated and produce various subset-specific cytokines that may contribute to both pathogen clearance and tissue repair. Moreover, we found that cells of the ILC2 subset progressively display common features with ILC1 during the infection time course, suggesting cell plasticity. We now wish to characterize the mechanisms controlling ILC2 dynamics and plasticity in the lungs, as well as their role in modulating TB pathology and anti-mycobacterial immunity.

P.D2.01.05
Dasatinib skews in vitro human CD56$^+$ innate lymphoid cells differentiation towards ILC3

***L. Damele**[1], E. Montaldo[2], L. Moretta[3], M. Mingari[4], C. Vitale[5];*
[1]CEBR-(Centre of Excellence for Biomedical Research), Italy, Genova, Italy, [2]IRCCS G. Gaslini, Genova Italy, present adress: San Raffaele Telethon Institute for Gene Therapy, IRCCS-San Raffaele Scientific Institute, Milano, Italy, [3]Immunology Area, Pediatric Hospital Bambino Gesù, Roma, Italy, [4]Dimes (Dipartimento Medicina Sperimentale), Università degli Studi di Genova, CEBR (Centre of Excellence for Biomedical Research), Università degli Studi di Genova, Policlinico San Martino, IRCCS indirizzo Oncologico, Genova, Italy, [5]Dimes (Dipartimento Medicina Sperimentale), Università degli Studi di Genova, Policlinico San Martino, IRCCS indirizzo Oncologico, Genova, Italy.

Tyrosine kinase inhibitors (TKI) improved the prognosis of Chronic Myeloid Leukemia and of Philadelphia$^+$ Acute Lymphoblastic Leukemia patients. However, TKI can develop of resistance and lack of complete molecular remission in the majority of patients. Clinical evidences suggest that the terminally differentiated CD56+CD16+CD57+ NK cells, may be associated with successful Imatinib therapy discontinuation or with a deep molecular response in Dasatinib-treated patients. Therefore, is important study how the chronic exposure to TKI may influence NK cell development and repertoire. CD34$^+$ Hematopoietic Stem Cells were cultured with appropriate cytokines to induce in vitro NK cell differentiation, in the absence or in the presence of Imatinib, Nilotinib, Dasatinib, or KX2-391 Src kinase inhibitor. At different time intervals, cells were counted and analyzed for the expression of lineage-specific markers, cytokines production and cytolytic activity. Our results show that Dasatinib sharply skewed the repertoire of CD56$^+$ cells, leading to a reduced recovery of CD56$^+$CD117$^-$CD16$^+$CD94/NKG2A$^+$EOMES$^+$ NK cells, to an impaired cytotoxicity, while IFN-γ production was increased. On the other hand, the development of CD56$^+$CD117$^+$CD94/NKG2A$^-$RORγt$^+$IL-22-producing ILC3 was not affected. This effect appears to involve the Dasatinib-mediated inhibition of Src kinases, as suggested by experiments with a KX2-391. Our experiments, revealed a possible mechanism by which Dasatinib may interfere with the maturation of competent NK cells, affecting signaling pathways required for differentiation and survival NK cell but not for of ILC3. Moreover, could identify new tools to design patients-individualized timing and dosing of Dasatinib to favor an NK-mediated immune response against leukemia.

P.D2.01.06
Characterization of CD56^{bright} NK cells which inversely correlate with survival of melanoma patients

***K. de Jonge**[1], A. Ebering[1], S. Nassiri[1,2], P. Baumgärtner[1], D. E. Speiser[1,3];*
[1]Dept. of Fundamental Oncology and Ludwig Cancer Center, Epalinges, Switzerland, [2]Swiss Institute of Bioinformatics (SIB), Lausanne, Switzerland, [3]Dept. of Oncology, University Hospital Center (CHUV), Lausanne, Switzerland.

The roles of NK cells in human melanoma remain only partially understood. We characterized NK cells from PBMC and found that their frequencies were similar between stage III/IV melanoma patients and healthy donors. Interestingly, overall survival was significantly reduced in the patients who had high frequencies of CD56^{bright} NK cells. The production of IFNγ, granzyme B, perforin and CCL4 by the CD56^{bright} NK cells from patients was equal to healthy controls. In contrast, we found lower production of TNFα, GMCSF and CCL3, suggesting that the CD56^{bright} NK cells might be less capable to fight against tumors. Furthermore, the increased expression of CD11a and CD38 may be associated with enhanced inhibition of T cells. Thus, the activation status of CD56^{bright} NK cells may contribute to poor clinical outcome by both reduced anti-tumor activity and increased T cell inhibition. Our results highlight the significance of CD56^{bright} NK cells in patient prognosis, emphasizing the potential of NK cells for biomarker discovery and future therapeutic targeting.

P.D2.01.07
Innate lymphocytes (ILCs) respond to crystal-deposition and promote local inflammation and fibrosis

***T. Frasconi**[1], S. Schwab[1,2], C. Kurts[1], I. Ludwig-Portugall[1];*
[1]Institute od Experimental Immunology, Bonn, Germany, [2]University Clinic, Department of Internal Medicine, Bonn, Germany.

Crystal-induced inflammation is a common manifestation of a variety of genetic and acquired metabolic disturbances. DCs or macrophages sense the crystals, activate the NLRP3-inflammasome and produce cytokines like IL18 and IL1β. These, together with others like IL23, promote local inflammation and induce the development of kidney fibrosis.
Our preliminary data suggested that a Roryt+ and RAG-independent population play an important role in the development of kidney fibrosis. Based on this,we aim to understand the mechanism by which ILCs sense and respond to crystal deposition and influence the development kidney fibrosis.
We use a mouse model of crystal nephropathy induced by adenine-enriched chow to analyze the cytokines profile of Rorγt+ and Rorγt- ILCs. We established an intra-renal injection of IL18, IL1β and IL23 to evaluate their effect on ILCs by analysis of cytokine production and cell proliferation. IL18, IL1β and IL23 are produced in crystal nephropathies by resident DCs and macrophages.
ILCs can sense this inflammatory environment and respond to these stimuli by releasing pro-inflammatory cytokines, such as IL17 and IFNγ, which promote the development of kidney fibrosis. The IFNγ production seems to be sustained not only by T-bet+ILC1, but also by ILC3.
Our data suggest that the pro-inflammatory environment promoted by crystal deposition has an effect on ILCs, which play a role in IFNγ and IL17 production suggesting plasticity within ILCs groups.
As a consequence, ILCs can be considered as early responders in a context of crystal nephropathies and might serve as a key player in kidney fibrosis.

P.D2.01.08
Type 3 Innate Lymphoid Cells might have an impact in active Behçet's Disease

***M. GELMEZ**[1], G. Ozcit[1], E. Cetin[1], S. Cinar[1], M. Erdugan[2], G. Babuna[3], A. Gul[2], A. Akdag-Kose[3], G. Deniz[1];*
[1]Aziz Sancar Institute of Experimental Medicine, Department of Immunology, Istanbul, Turkey, [2]Istanbul University, Istanbul Faculty of Medicine, Department of Rheumatology,, Istanbul, Turkey, [3]Istanbul University, Istanbul Faculty of Medicine, Department of Dermatology, Istanbul, Turkey.

Innate lymphoid cells (ILCs) are lymphoid cells that do not express rearranged receptors and have important effectors and regulatory functions in innate immunity and tissue remodeling. Uncontrolled activation and proliferation of ILCs can contribute to inflammatory autoimmune diseases. Behcet's disease (BD) is a complex systemic inflammatory disorder and the role of ILCs is still unknown in BD. In this study, the quantity and effect of ILCs in the pathogenesis of BD were investigated. PBMC were isolated from whole blood from eleven BD patients (7 active and 4 inactive stage) and five healthy controls. ILCs were analyzed by using anti-Lineage, -CD45, -CD161, -CRTH2, -NKp44, -c-kit and -CD127 monoclonal antibodies by flow cytometry. Total ILC, ILC3 and NKp44$^-$ ILC3 cells were increased in all patients compared to healthy subjects (p= 0.05, p= 0.05, and p= 0.0066, respectively), whereas ILC1 and NKp44$^+$ ILC3 cells were decreased (p= 0.05, and p= 0.0066, respectively).

Similarly, CD161$^+$ cells (total ILC), ILC3 and NKp44$^-$ ILC3 cells were increased (p= 0.05, p= 0.0002, and p= 0.0002, respectively); ILC1 and NKp44$^+$ ILC3 cells were decreased (p= 0.01, and p= 0.0002, respectively) in active patients compared to healthy subjects, not in inactive patients. Increased neutrophilic infiltration and IL-17 secreting Th17 cells in BD were shown. It is known that ILC3$^+$ cells are similar to Th17 subset as cytokine variety and transcription factors, regarding our preliminary data showed that inflammatory status in BD might direct ILC cells to ILC3$^+$ subset.

P.D2.01.09
Innate lymphoid cells interact with fibroblastic reticular cells in lymphoid organs

***B. A. Heesters**, L. Krabbendam, S. M. Bal, H. Spits;*
AMC, Amsterdam, Netherlands.

Fibroblastic Reticular Cells (FRCs) together with other lymph node stromal cells (LNSCs), regulate immunity and self-tolerance, yet key aspects of their biology remain ill defined. FRCs are located within lymphoid organs and organize the T cell zone. They are strategically localized to respond quickly to dangerous stimuli. FRC are well equipped to detect danger signals and are able to respond by secretion of cytokines long before the onset of adaptive immunity. Innate lymphoid cells (ILCs) readily respond to cytokines early during inflammation. FRCs could be a source of these activating cytokines. However, ILCs also require cytokines to survive and to retain the phenotype required in lymphoid tissues. Here we show that FRCs are responsible for ILC survival and differentiation within lymphoid organs. FRCs are a source of interleukin (IL)-7, IL-4 and various other cytokines and chemokines. Besides secretion of cytokines vital for ILC survival, FRCs express a plethora of cytokine and chemokine receptors. These discoveries led to the development of an FRC cell line that supports ILC survival and differentiation in vitro. This culture system allows for in vitro ILC experiments that more closely resemble the situation in vivo. Together our data describe the reciprocal relationship between FRC and ILC in lymphoid organs.

P.D2.01.10
Type 2 Innate lymphoid cells are increased cells in acute dengue infections

***A. I. Kamaladasa**[1], Y. Perera[1], L. Gomes[1], D. Jayathilake[1], C. Hardman[2], A. Wijewickrama[3], G. Ogg[2], N. Malagive[1,2];*
[1]Centre for Dengue Research,, Nugegoda, Sri Lanka, [2]MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, Oxford NIHR Biomedical Research Centre and University of Oxford, Oxford, United Kingdom, [3]Infectious Disease Hospital, Angoda, Sri Lanka.

Objective: Type 2 Innate lymphoid cells (ILC2s) are known to produce type 2 cytokines during viral infections in the mice models. During acute dengue viral (DENV) infections type 2 cytokines are elevated in individuals with severe disease. Therefore, we set out to investigate the possible role of ILC2s in the pathogenesis of acute dengue. **Methods:** The frequency of ILC2s were determined in adult patients with dengue fever (DF) (n=40) and dengue haemorrhagic fever (DHF) (n=26) and in and 15 healthy individuals. Serum IL-4, IL-5 and IL-13 were measured in serum samples taken daily throughout the course of illness in some of these patients (n=20). **Results:** The frequency of ILC2 in patients with DHF (median 0.6%, IQR 0.33 to 1.03%) were significantly higher (p=0.02) compared to healthy individuals (median 0.36% IQR 0.17 to 0.48%).
Further the absolute ILC2 count was significantly higher (P=0.03) individuals with DHF (median 8.52, IQR 5.74 to 14.54 cells/mm^3) compared to DF (median 0.52 , IQR 1.41 to11.14 cells/mm^3). However, there was no difference in the proportion of ILC2s in patients with DF compared to those with DHF. Serum IL-13 and IL-5 was not detected in any of the patients, while IL-4 was detected in 7 patients. **Conclusion:** As the proportion and absolute count of ILC2 in DHF is significantly increased these cells could be playing a role in DENV infections. But this role may not be related to Th2 cytokine production as we could not detect these cytokines in acute dengue patients.

P.D2.01.11
ILC2s regulate early anti-cryptococcal immunity

***M. Kindermann**[1], L. Knipfer[1], S. Obermeyer[2], U. Schleicher[2], C. Bogdan[2], G. Alber[3], M. Neurath[1], S. Wirtz[1];*
[1]Medical Department 1, Erlangen, Germany, [2]Microbiology, Erlangen, Germany, [3]Immunology, Leipzig, Germany.

Infections with the opportunistic pulmonary pathogen *Cryptococcus neoformans* are a leading cause of co-morbidity and mortality of immunocompromised individuals. Initially, pulmonary infection originates from inhaled spores or desiccated fungi. Clinical signs manifest ranging from mild asymptomatic nodular disease to severe acute respiratory distress syndrome (ARDS) cumulating in fungal dissemination to the CNS, which triggers fatal meningitis or meningoencephalitis.
The host immune status as well as the prominent type of immune response is decisive for the course of disease. Here, a type-2 biased immune response is rather linked to a poor prognosis whereas type-1/3 immunity is related to fungal control and clearance.
Despite knowing that type-2 immunity is detrimental during late stages of pulmonary cryptococcosis, little is known about early events directing subsequent immunomodulatory pathways.
In order to address this issue, we investigated ILC2-deficient mice during onset of infection since ILC2s play crucial roles in mediating early type-2 responses. Noteworthy, ILC2 deficient mice evolve a less prominent detrimental type-2 immunity indicated by reduced amounts of classical markers of type-2 immunity such as IL-4, IL-13 and AAM-markers. Contrary, type-1/3 immune response is increased in these mice indicated by elevated levels of type-1/3 signature cytokines. Consequently, this phenotypic shift results in more efficient fungal control accompanied by less severe lung pathology and prolonged survival.
In conclusion, we provide evidence that ILC2s function as early immune regulators during pulmonary cryptococcosis by favoring detrimental type-2 immunity. Further, intervening with this regulatory checkpoint may be a potential approach for anti-mycotic treatments in human patients.

P.D2.01.12
Identification of a human KLRG1$^+$ CRTH2$^{\pm}$ ILC2 precursor

***M. Nagasawa**, B. A. Heesters, C. M. Kradolfer, H. Spits, S. M. Bal;*
AMC, Amsterdam, Netherlands.

Group 2 Innate lymphoid cells (ILC2s) produce type 2 cytokines in response to epithelial cell derived cytokines such as thymic stromal lymphopoietin (TSLP) and interleukin 33 (IL-33). These cells are important in immune responses to helminths, but they can also be involved in the pathogenesis of inflammatory airway and skin diseases. Human ILC2s depend on the transcription factor GATA3 and express prostaglandin D2 receptor 2 (PTGDR2, also known as CRTH2). However, little is known about their development. To characterize human ILC2s in detail, we analyzed the phenotype and transcriptome of adult peripheral blood (PB) ILCs. In addition, ILC subsets isolated from PB were co-cultured with an OP9 mouse bone marrow stromal cell line to evaluate their developmental capacity and functionality. Here we identified a pre-ILC2 population that lacks CRTH2 expression, but does express the ILC2 associated molecule Killer cell lectin-like receptor subfamily G member 1 (KLRG1). Transcriptome analysis of KLRG1$^+$ CRTH2$^{\pm}$ ILCs suggested that these cells are ILC2 precursors. This notion is supported by the observation that KLRG1$^+$ CRTH2$^{\pm}$ ILCs could differentiate towards conventional CRTH2$^+$ ILC2s *in vitro*. In contrast to conventional ILC2s, pre-ILC2s also produce type 1 (IFN-γ) and type 3 (IL-17 and IL-22) cytokines upon stimulation with IL-1β and IL-23. This suggests these cells are of a developmentally immature stage and have more functional flexibility than conventional ILC2s.

P.D2.01.13
MESENCHYMAL STROMAL CELLS STIMULATE THE PROLIFERATION AND INTERLEUKIN-22 PRODUCTION OF GROUP 3 INNATE LYMPHOID CELLS

***S. Z. Omar**[1,2,3], V. v. Hoeven[1,2,3], M. Munneke[1,2,3], A. S. Cornelissen[4], M. J. Spruit[1], M. Kleijer[4], J. H. Bernink[2,3], B. Blom[2,3], C. Voermans[4], M. D. Hazenberg[1,2,3];*
[1]Department of Hematology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands, [2]Department of Experimental Immunology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands, [3]Cancer Center Amsterdam and Amsterdam Infection and Immunity Institute, Amsterdam, Netherlands, [4]Department of Hematopoiesis, Sanquin Research and Landsteiner Laboratory, Amsterdam, Netherlands.

Infusion of mesenchymal stromal cells (MSCs) is a promising and increasingly applied therapy for patients who suffer from a variety of inflammatory diseases, including graft-versus-host disease (GvHD), a common and life-threatening complication after allogeneic hematopoetic stem cell transplantation (aHSCT). The therapeutic effect of MSCs is mainly ascribed to their ability to suppress the activity of (alloreactive) T cells and to support tissue-repair. However, clinical response rates in patients with GvHD are limited to 50%, and the determinants for MSC responsiveness are unknown. We demonstrated that high frequencies of activated group 3 innate lymphoid cells (ILC3s) before and after aHSCT were associated with a lower risk of GvHD. This may be related to interleukin (IL)-22 production by ILC3s, a cytokine important for intestinal epithelial cell homeostasis. To Study whether ILC3s may contribute to the therapeutic effect of MSCs, we co-cultured human tonsil-derived ILC3s with human bone-marrow derived MSCs in standard or transwell culture plates. In co-cultures, MSCs stimulated the proliferation and IL-22 production of ILC3s and reciprocally, ILC enhanced MSC expression of VCAM-1 and ICAM-1. In both directions the stimulatory effect was mainly mediated by cell-cell contact. Experiments with various blocking agents revealed that MSC derived soluble factors, namely IL-7 and aryl hydrocarbon receptor ligands, also stimulate ILC3 proliferation and function. Thus, in addition to inhibiting the proliferation of alloreactive T cells, MSCs also promote the expansion and IL-22 production of ILC3s which may be important for the treatment of various inflammatory conditions in the intestine, including GvHD.

P.D2.01.14

First-breath-induced type 2 pathways shape the lung immune environment

S. Saluzzo[1,2,3], *A. Gorki*[1,2], *B. M. Rana*[3], *R. Martins*[1,2], *S. Scanlon*[3], *P. Starkl*[1,2], *K. Lakovits*[1,2], *A. Hladik*[1,2], *A. Korosec*[1,2], *O. Sharif*[1,2], *J. M. Warszawska*[1,2], *H. Jolin*[3], *I. Mesteri*[4], *A. N. McKenzie*[3], *S. Knapp*[1,2];
[1]*Medical University of Vienna, Vienna, Austria,* [2]*CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria,* [3]*MRC Laboratory of Molecular Biology, Cambridge, United Kingdom,* [4]*Institute of Pathology Überlingen, Überlingen, Germany.*

From birth onward, the lungs are exposed to the external environment and therefore harbor a complex immunological milieu to protect this organ from damage and infection. We investigated the homeostatic role of the epithelium-derived alarmin interleukin-33 (IL-33) in newborn mice and discovered the immediate upregulation of IL-33 from the first day of life, closely followed by a wave of IL-13- producing type 2 innate lymphoid cells (ILC2s), which coincided with the appearance of alveolar macrophages (AMs) and their early polarization to an IL-13-dependent anti-inflammatory M2 phenotype. ILC2s contributed to lung quiescence in homeostasis by polarizing tissue resident AMs and induced an M2 phenotype in transplanted macrophage progenitors. ILC2s continued to maintain the M2 AM phenotype during adult life at the cost of a delayed response to Streptococcus pneumoniae infection in mice. These data highlight the homeostatic role of ILC2s in setting the activation threshold in the lung and underline their implications in anti-bacterial defenses.

P.D2.01.17

Transcriptional regulation of human natural killer cell and ILC3 development and function by ETS-1

S. Taveirne[1], *S. Wahlen*[1], *L. Kiekens*[1], *E. Van Ammel*[1], *K. De Mulder*[1], *J. Roels*[1], *L. Tilleman*[1], *M. Aumercier*[2], *F. Van Nieuwerburgh*[1], *T. Kerre*[1], *T. Taghon*[1], *B. Vandekerckhove*[1], *G. Leclercq*[1];
[1]*Ghent University, Ghent, Belgium,* [2]*Université de Lille, Lille, France.*

Introduction: Natural killer (NK) cells and innate lymphoid type 3 cells (ILC3s) are innate lymphoid cells that play a critical role in the immune response against tumor cells and pathogens, and also have important immune regulatory functions. The transcription factor Ets-1 is an important factor in murine NK cell biology as Ets-1-deficient mice show severely reduced NK cell numbers and residual NK cells display decreased functionality. No data regarding the function of ETS-1 in the development of human NK cells is available. In addition, the role of ETS-1 in neither murine nor human ILC3 biology has been investigated.
Methods: In this study, we generated ETS-1-deficient human embryonic stem cell (hESC) clones using the CRISPR/Cas9 technology. In a complementary approach, we generated ETS-1 loss-of-function cord blood hematopoietic stem cells (HSCs) by retroviral transduction of the dominant-negative ETS-1 p27 isoform.
Results: Each ETS-1-deficient human ESC clone displayed defective NK cell differentiation capacity. ETS-1 p27-transduced HSCs had a lower potential to differentiate into NK cells and NKp44$^+$ ILC3, which correlated with increased apoptosis. Residual NK cells showed reduced cytokine secretion and cytotoxic activity. Transcriptome analysis showed that ETS-1 had a dual role in NK cells and NKp44$^+$ ILC3, as it both induces and inhibits expression of NK cell- and ILC3-specific genes, respectively.
Conclusion: Our data show that ETS-1 is a critical regulator of human NK cell and ILC3 development and function and provide important insights in the molecular mechanisms of their biology.

P.D2.01.18

ILC3 NCR$^+$ regulate endothelial cell activation through NF-kB

G. Vanoni, *P. Romero, S. Trabanelli, C. Jandus;*
University of Lausanne, Lausanne, Switzerland.

Innate lymphoid cells (ILCs) represent the most recently identified subset of lymphocytes. Despite their established involvement in inflammatory immune responses, the role of ILCs in cancer remains poorly defined.
Our aim is to assess whether ILCs might exert an active role in controlling or promoting tumor growth through the interaction with the endothelium.
Therefore, short-term in vitro expanded ILC subsets isolated from the peripheral blood of healthy donors were used in 3h co-culture experiments with an endothelial cell line (HUVEC, human umbilical vascular endothelial cell line) at 1:1 ratio. The activation state of endothelial cells (ECs) was assessed by flow cytometry, by evaluating the level of surface expression of the adhesion molecules E-Selectin, ICAM-1 and VCAM-1.
Among all ILC subsets, ILC3 NCR$^+$ elicited the strongest upregulation of adhesion molecules in ECs, in a contact-dependent manner. By specifically blocking the NF-kB pathway in ECs, the level of expression of adhesion molecules was reverted to basal levels. Pre-exposure of ILC3 NCR$^+$ to human bladder carcinoma cell lines strongly impaired this capacity. ILC3 NCR$^+$ induce the expression of adhesion molecules in ECs via NF-kB pathway. The in vitro ECs-ILCs interaction will be further evaluated to assess its functionality and to identify the molecular players. With the use of tumor-bearing mice, the in vivo relevance of the in vitro findings will be tested to unravel if this capacity of ILC3 NCR$^+$ could represent a way for facilitating the immune cell infiltration in the tumor and, therefore, impact tumor progression and/or growth.

P.D2.01.19

Relationship between group 3 innate lymphoid cells (ILC3) and Th17 in human nasopharynx-associated lymphoid tissue and their association with pneumococcal carriage in humans

L. Zaki[1], *R. Xu*[1], *M. S. Ahmed*[1], *R. Sharma*[2], *S. Leong*[3], *N. French*[1], ***Q. Zhang***[1];
[1]*University of Liverpool, Liverpool, United Kingdom,* [2]*Alder Hey Children's Hospital, Liverpool, United Kingdom,* [3]*Aintree University Hospital, Liverpool, United Kingdom.*

Innate lymphoid cells including ILC3 are increasingly appreciated as being critical in local immune homeostasis and inflammation. Similar to Th17 cells, ILC3 express IL17A and/or IL22. Recent data from animal models suggest there is a reciprocal interaction between ILC3 and T cell responses. It is not known whether any relationship between ILC3 and Th17 cells in human nasopharynx and whether ILC3 contributes to the regulation of pneumococcal carriage in humans. Methods: We have studied the ILC3 and Th17 populations in the nasopharynx-associated lymphoid tissue (NALT) from children and adults following stimulation by PMA or a Staphylococcal extract, and analysed their association n with pneumococcal carriage. ILC3 and Th17 frequencies and responses following stimulation were examined by flow-cytometry following staining for linage markers, CD127, NKP44, c-kit, RORgt, IL17A and IL-22. Results: We showed the ILC3 frequency in NALT was higher in children and mainly express IL22 which was in contrast to the markedly higher Th17 frequency in adults. Further analysis revealed that there was an inverse relationship between the IL22-expressing ILC3 and IL17A- producing Th17 cells, and a higher frequency of ILC3 was shown with pneumococcal carriage positive than in negative children. *S.aureus* stimulation reduced ILC3 number and HLA-DR expression but markedly increased Th17 response. *S.aureus* induced Th17 response was inhibited by RORgt-blocker which correlated to an increase in ILC3. Our results suggest there is significant interactions between ILC3 and pathogen-induced Th17 response, and ILC3 may critically regulate Th17 response in human nasopharynx through which mediate bacterial carriage in children.

P.D2.01.20

HIV-1 Infects and depletes innate lymphoid cells via type I interferon pathway

J. Zhao[1], *L. Cheng*[2], *L. Su*[2], ***Z. Zhang***[1];
[1]*Beijing 302 hospital, Beijing, China,* [2]*University of North Carolina, Chapel Hill, United States.*

Innate lymphoid cells (ILCs), including ILC1, ILC2 and ILC3 subsets, have emerged as central players in homeostatic and inflammatory conditions, and correlated with the pathogenesis of multiple human diseases. Our recent studies found that ILC3s were severely depleted from gut mucosal of patients with chronic HIV-1 infection via Fas/FasL-mediated pathway. Blockade of type I interferon (IFN-I) pathway significantly restored ILC3 loss in humanized mice with HIV-1 infection. However, it is not clear whether HIV-1 can infect ILCs. Here, we found that human ILC1s comprising of CD4+ and CD4- subpopulations were present in various human lymphoid organs but with different transcription programs and functions. CD4+ ILC1s expressed HIV-1 co-receptors and were productively infected by HIV-1 *in vitro* and *in vivo*. HIV-1 infection leads to activation, depletion and functional impairment of ILC1sin humans and in humanized mice*in vivo*. Highly active antiretroviral therapy (HAART) efficiently rescued the ILC1 numbers and reduced their activation, but failed to restore their functionality in HIV-1 patients. Blocking IFN-I signaling also prevented HIV-1 induced depletion or apoptosis of ILC1 cellsin vitro and in humanized mice in vivoduring HIV-1 infection. Our study identified the CD4+ ILC1 cells as a new target population for HIV-1 infection, and revealed that IFN-I contributes to the depletion of ILC1s during HIV-1 infection.

P.D2.02 NK cells and innate immune mechanisms

P.D2.02.01
CARMIL2 splice site mutation in a patient with warts

F. Atschekzei[1], *R. Jacobs*[1], *G. Ahrenstorf*[1], *A. Dhingra*[2], *R. Schmidt*[1];
[1]*MHH, Department of Clinical Immunology and Rheumatology, Hannover, Germany,* [2]*MHH, Department of Virology, Hannover, Germany.*

We report a novel homozygous spice site mutation in *CARMIL2* (*RLTPR*) gene underlying a primary immunodeficiency with warts.Molecular genetic testing was composed of targeted next-generation sequencing of a panel of PID related genes. A now 29-year-old male patient, born of consanguineous parents, presented since childhood with a history of eczema, recurrent bacterial infections such as pyoderma and pneumonia. Since the age of 22 he suffers from disseminated and persistent warts (verruca vulgaris palmaris) particularly in the hands and sole of the feet. Targeted next generation sequencing identified a novel homozygous splice-acceptor site mutation [c.795-1 G>A] in *CARMIL2* gene, which co-segregated with the disease phenotype. This is one of few reports of cutaneous warts associated with a homozygous mutation in *CARMIL2* which underlines the role of CARMIL2 in primary immunodeficiency. Therefore, it might be considered in the molecular differential diagnosis of patients with disseminated and/or persistent warts. Supported by DZIF TTU 07.801

P.D2.02.02
Tumor necrosis factor superfamily member induces Ig synthesis against PAMP stimulation by activatingNF-κB pathway in freshwater carp, *Catla catla*

R. Banerjee[1], *M. Samanta*[2], *S. Das*[1];
[1]*National Institute of Technology, Rourkela, India,* [2]*ICAR- Central Institute of Freshwater Aquaculture, Bhubaneswar, India.*

Introduction: Tumor necrosis factor superfamily (TNFSF) members consist of different cytokines amongst which, B cell activating factor (BAFF) and a-proliferation-inducing ligand (APRIL) are involved in B-cell development playing a crucial role in adaptive immunity. These two similar homology domain ligands have three common receptors: BAFF receptor, BCMA and TACI which binds to enhance the immune system in wide range of vertebrates. Therefore, to gain insights into the efficacy of Ig synthesis in the Indian Major Carp *Catla catla*, the binding of BAFF/APRIL to the receptors were studied which may trigger the immune system in downstream signaling cascade orchestrating the Ig synthesis.
Materials and Methods: The immune response of BAFF and APRIL along with downstream signaling molecules involved in NF-κB pathway have been analysed by performing qRT-PCR, immunoblotting and flow cytometry. Further, NF-κB inhibitor was also used to inhibit the NF-κB complex to investigate the transcriptomic analysis of the signaling molecules along with Ig synthesis.
Results: The qRT-PCR analysis elucidated their role in PAMPs stimulated tissues leading to the upregulation and activation of downstream signaling molecules. The leukocyte isolated from the blood was treated with r-BAFF which showed an enhancement in the Ig synthesis.
Conclusion: The study can therefore prove the insight of BAFF and APRIL during PAMPs stimulation in carps augmenting the Ig synthesis by NF-κB dependent pathway. Expressions of r-BAFF also showed the critical role in adaptive defence mechanism in *C. catla*.
Grants and fellowships: NASF, ICAR, Govt of India (Project code BS/4003) is acknowledged for financial support.

P.D2.02.03
Long term effects of chronic HCV infection on Natural Killer cells

W. Bi[1], *M. Heyner*[1], *J. Hengst*[2], *L. Nilse*[3], *B. Bulitta*[1], *F. Klawonn*[1,4], *H. Wedemeyer*[2], *L. Jänsch*[1];
[1]*Helmholtz Center for Infection Research, Braunschweig, Germany,* [2]*Hannover Medical School, Hannover, Germany,* [3]*University of Freiburg, Freiburg, Germany,* [4]*Ostfalia University of Applied Sciences, Wolfenbüttel, Germany.*

Natural killer (NK) cells play a pivotal role in the direct defense against tumors and viral infections. Basically, NK cells have the capacity to counteract hepatitis C virus (HCV) infections. However, NK immune responses are apparently impaired in chronically infected HCV patients, which contribute to HCV-related diseases including hepatocellular-carcinoma (HCC). Since 2014, direct acting antivirals (DAAs) such as Harvoni®, were approved for therapy of HCV patients. While DAA application results in complete viral clearance, it was tempting to speculate that HCV-mediated immune evasion mechanism in NK cells are rescued in parallel. This project aims to decipher mechanisms of how HCV infections cause NK cell dysfunctions. We collected primary NK cells from Harvoni®-treated HCV patients before, along and after therapy, and then performed a comprehensive molecular phenotyping by means of quantitative proteomics. We found important NK cell functions from cured patients still de-regulated after virus clearance suggesting long-term effects on human NK cell immunity. Among those functions, we selected ADAP to complement information about its importance for human NK cell responses. We newly generated ADAP knock out NK cells and performed *in vitro* assays that determined its role in cytotoxicity, degranulation and cytokine production. This project is supported by China Scholarship Council.

P.D2.02.04
Persistent replication of HIV, HCV and HBV results in distinct gene expression profiles by human NK cells

L. Boeijen, *J. Hou, R. de Groen, A. Verbon, A. Boonstra;*
Erasmus MC, Rotterdam, Netherlands.

Background: NK cells during chronic viral infection have been well studied over the last decade. We performed an unbiased next-generation RNA-sequencing approach to identify commonalities or differences of the effect of HIV, HCV and HBV viremia on NK cell transcriptomes.
Methods: Using cell sorting, we obtained CD3-CD56+ NK cells from blood of 6 HIV, 11 HCV, and 32 HBV infected patients without treatment. After library preparation and sequencing, we used an in-house analytic pipeline to compare expression levels with matched healthy controls.
Results: In NK cells from HIV, HCV and HBV patients, transcriptome analysis identified 272, 53, and 56 differentially expressed genes, respectively (fold change >1.5, q-value 0.2). Interferon stimulated genes were induced in NK cells from HIV/HCV patients, but not during HBV infection. HIV viremia downregulated ribosome assembly genes in NK cells. In HBV, viral load and ALT variation had little effect on genes related to NK effector function.
Conclusion: We compare, for the first time, NK cell transcripts of viremic HIV, HCV and HBV patients. We clearly demonstrate distinctive NK cell gene signatures in 3 different populations, suggestive for a different degree of functional alterations of the NK cell compartment as compared to healthy individuals.

P.D2.02.05
Mature NKG2C$^+$ NK cells demonstrate increased HLA-DR expression

S. A. Erokhina, *P. A. Kobyzeva, M. A. Streltsova, E. I. Kovalenko;*
Institute of Bioorganic Chemistry of RAS, Moscow, Russian Federation.

A common reaction of human NK cells to CMV infection is appearance of highly differentiated CD56^{dim}NKG2C$^+$NKG2A$^-$CD57$^+$ subset, often described as a variation of “adaptive” NK cells. The activating receptor NKG2C can be also found on the surface of less differentiated CD56^{dim}CD57$^-$ and CD56^{bright} cells. In order to investigate similarities and distinctions of the different NKG2C-positive subsets we have analyzed by flow cytometry the surface expression of HLA-DR, known as a marker of NK cell activation, in NK cells at certain differentiation stages.
In *ex vivo* studies, we observed significant proportion of HLA-DR-expressing cells among all NKG2C-positive NK cells. In CD56^{dim}CD57$^+$ and CD56^{dim}CD57$^-$ subsets, NKG2C$^+$ NK cells demonstrated higher level of HLA-DR-positive cells, along with higher HLA-DR expression intensity, than respective NKG2C$^-$ cells, whereas in CD56^{bright} subset HLA-DR expression level in NKG2C$^+$ and NKG2C$^-$ NK cells were similar. After in vitro stimulation of bulk cultures with IL-2 and K562 feeder cells expressing membrane-bound IL-21, the proportion of HLA-DR$^+$ cells increased in all NK cells, but especially in NKG2C-positive. Besides, among clonally expanded NK cell populations, acquired in the same stimulating conditions, HLA-DR expression intensity was higher in clones from NKG2C$^+$CD56^{dim}CD57$^-$/ NKG2C$^+$CD56^{dim}CD57$^+$ subsets compared to clones from their NKG2C$^-$ counterparts.
Thus, *in vivo* part of NKG2C$^+$ NK cells, but not NKG2C$^-$ NK cells, from more differentiated CD56^{dim} subset maintain HLA-DR expression, demonstrating more activated status than NKG2C$^-$ cells, and the same NK cells respond more effectively to cytokine/feeder cells stimulation.
The work was supported by Russian Science Foundation, grant #16-15-00309.

P.D2.02.06
Endometrial natural killer cells reveal a tissue-specific receptor repertoire

D. Feyaerts[1], *T. Kuret*[1], *B. van Cranenbroek*[1], *S. van der Zeeuw-Hingrez*[1], *O. W. van der Heijden*[2], *A. van der Meer*[1], *I. Joosten*[1], *R. G. van der Molen*[1];
[1]*Department of Laboratory Medicine, Laboratory for Medical Immunology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands,* [2]*Department of Obstetrics and Gynaecology, Radboud University Medical Center, Nijmegen, Netherlands.*

Natural killer cells (NK) discriminate foreign and malignant cells from healthy ‘self’ cells. However, during pregnancy local uterine NK cells play a different and more regulatory role by creating an immunologically privileged niche, and promoting trophoblast invasion and correct placenta formation. NK receptors (NKRs) are central regulators of NK activity, however, little is known about NKR expression on pre-pregnancy endometrial NK cells (eNKs). In order to bridge this knowledge gap, we analyzed NKR expression on pre-implantation eNKs to examine their potential role in successful pregnancy and questioned whether the NKR repertoire of eNK cells is different from peripheral blood NK cells (pbNKs).
eNK cells, obtained from menstrual blood, were immunophenotyped for NKR expression using flow cytometry, and compared to pbNKs of the same female.

Results showed that the NKR repertoire of eNKs is distinct from pbNKs, with eNKs co-expressing more than 3 NKR simultaneously. In contrast to the pbNKs, expansions of NK subpopulations present in the eNKs were independent of prior cytomegalovirus (CMV) infection and *HLA-C* genotype, suggesting rapid local turnover of eNKs and/or a distinct licensing process. Taken together, our data reveals that eNKs have a unique tissue-specific signature, suggesting they are finely tuned to accept the semi-allogenic fetus. These findings pave the way for the evaluation of eNK function during pregnancy complications, and may yield insight into their pathogenesis, thereby setting the stage for the discovery of pregnancy-success related biomarkers.

P.D2.02.07

Early Extracellular Followed by a Late Surge in Intracellular Release of Oxygen Radicals in Rat Neutrophils Following Acute Burn Injury With Sepsis

*N. Fazal, **M. Ibrahim**;*
Chicago State University, Chicago, United States.

Background: This is a study of neutrophil host defense and neutrophil oxidant production in burn-injured rats with a superimposed E. faecalis infection; it will enhance our understanding of pathogenic mechanisms by which infections exacerbate host defense dysfunction occurring with burn injury alone.
Methods: Our studies focus on alterations in neutrophils' oxidative in burn rats inoculated with Enterococcus faecalis. Blood and peritoneal PMN were obtained from different experimental and control groups of animals. O2- production both extracellular and intracellular was measured in PMN before and after their stimulation with PMA (100ng/mL) using Isoluminol-enhanced luminometry.
Results: We found that there is an early extracellular followed by a late surge in intracellular release of oxygen radicals in rat neutrophils following acute burn injury with sepsis. Blood PMN O2- remained upregulated after infection of rats with E. faecalis superimposed with the burn injury. E. faecalis alone did not cause an increase in blood PMN O2- to the level caused by burn alone. E. faecalis plus burn cause a much greater increase in peritoneal PMN O2- than in blood PMN O2- compared to the increase with burn alone.
Conclusions: These data show tissue PMNs exiting into the peritoneal cavity are activated to extracellularly release greater quantities of O2- than PMNs in blood with the combined burn and E. faecalis injury. Thus there was a likely exacerbation of extracellular O2- release by tissue PMNs in the combined injury. We conclude a differential kinetics of ROS release in circulatory and tissue neutrophils following burn and sepsis.

P.D2.02.08

CD127+ Innate Lymphoid Cells (ILCs) with NK cell features accumulate in Inflammatory Bowel Disease (IBD)

***L. Krabbendam**[1], C. Kradolfer[1], C. Buskens[1], W. Bemelman[1], J. H. Bernink[2,1], H. Spits[1];*
[1]Academic Medical Center, Amsterdam, Netherlands, [2]Hubrecht Institute, Utrecht, Netherlands.

Crohn's disease and Ulcerative Colitis are intestinal inflammatory disorders characterized by dysregulated immune responses. Innate Lymphoid Cells (ILCs), including helper ILCs and NK cells, are important for intestinal homeostasis and protective immune responses, but can contribute to inflammatory diseases when not properly balanced. Three helper ILC subsets are distinguished by their cytokine profile but each subset adapts its phenotype and cytokine profile in response to environmental cues. This plasticity is extensively described between helper ILC subsets, but the distinction and plasticity between helper ILCs and NK cells requires further investigation.
In human intestinal tissue, we identified an IFN-γ-producing cell type that expresses the ILC markers CD127 (IL-7R) and the NK cell marker CD94. Based on phenotype, transcription profile and cytokine secretion this cell type resembles both ILCs and NK cells. *In vitro* experiments demonstrated that conventional helper ILCs acquire a cytotoxic, IFN-γ-producing CD94$^+$ phenotype when exposed to IL-12 that can kill K562 target cells. Since these cytotoxic ILCs retain expression of a number of ILC markers and lack some NK markers they remain distinct from NK cells. This indicates that helper ILCs can acquire NK cell features, which may be reversible. Thus, like CD4+ T cells, helper ILCs can acquire features of cytotoxic cells. These cytotoxic CD127+CD94+ ILCs are more prominent in human adult inflamed intestine compared to non-inflamed resection specimen and virtually absent in human fetal intestine, suggesting that an inflammatory environment favors accumulation of this cell type. Therefore, these novel cytotoxic CD127+CD94+ ILC may contribute to IBD pathology.

P.D2.02.09

Murine gamma delta T cells display distinct subpopulations based on their ability to phagocytose unopsonised bacteria

***J. C. Lenzo**, S. Fong, J. Holden, N. M. O'Brien-Simpson, E. C. Reynolds;*
Oral Health Cooperative Research Centre, Melbourne Dental School, Bio21 Institute, The University of Melbourne, Melbourne, Victoria, Australia., Melbourne, Australia.

In humans and mice, gamma-delta (γδ) T cells constitute approximately 1-5% of the total circulating T cells. Despite being a minority in circulation, murine γδ T cells exist as a large proportion in skin and mucosal sites. Given that mucosal epithelium serves as the initial barrier against invading pathogens, roles in immune regulation and infection surveillance are proposed for γδ T cells. Chronic periodontitis, an inflammatory disease of the supportive tissues of the teeth, leads to resorption of alveolar bone and eventual tooth loss. Although chronic periodontits is associated with a polymicobial biofilm, specific bacterial species such as *Porphyromonas gingivalis* are closely associated with clinical measures of disease. Using *in vivo* mouse models, we show that γδ T cells respond rapidly upon *P. gingivalis* infection and are able to phagocytose unopsonised *P. gingivalis*, confirming their role in innate immune responses. We have also identified novel murine γδ T cell subpopulations based on cell surface marker expression and ability to phagocytose bacteria, CD27$^-$ γδ T cells are efficient phagocytes while CD27$^+$ γδ T cells are less phagocytic and express higher levels of expressed antigen presentation markers. We have also found that human blood γδ T cells can phagocytose unopsonised *P. gingivalis*, indicating that they may also play a role in the immune response observed in human periodontitis. Based on our findings, we propose that the slower phagocytic CD27$^+$ γδ T cells have a more antigen presentation role, whereas the rapid phagocytic CD27$^-$ γδ T have a more microbial clearance role.

P.D2.02.10

Molecular functional characterisation of neutrophil granulocytes during *Aspergillus fumigatus* infection

***F. Neumann**[1], J. Weski[1], P. Seddigh[1], T. Bracht[2], M. Gunzer[1];*
[1]University Hospital Essen, Institute for Experimental Immunology and Imaging, Essen, Germany, [2]Ruhr-Universität Bochum, Medizinisches Proteom-Center, Bochum, Germany.

Invasive Aspergillosis is common condition in individuals suffering from one of various forms of immune suppression. The cause of this ailment are inhaled conidia of the mould fungus *Aspergillus fumigatus*, normally cleared from the airways predominantly by neutrophil granulocytes. If the clearing fails, the conidia start to germinate and the hyphal network can spread and invade every mayor organ. However, the underlying mechanisms making or breaking a successful clearance are still to be identified. By analysing the proteome of neutrophils from mice challenged with conidia, we could identify proteins which are highly regulated in the activated cell, like PAD4 or MPO. The majority of proteins we identified remain as of yet uncharacterised in an infectious context and are now investigated utilizing immortalised HoxB8 progenitor cells. We knock out the correlating genes via CRISPR/Cas and the *in vitro* differentiated HoxB8 neutrophils will be examined in an array of methods designed to assess neutrophil functionality, e.g. NET formation or ROS production. We created HoxB8 cells which express tdTomato only when differentiated into neutrophils. This specific fluorescence permits us to confirm gene function of promising candidates even under near physiological conditions by transferring knockout cells into mice and tracking them to verify their role in an *in vivo* model of pulmonary aspergillosis. In researching the basic mechanisms of the mostly unnoticed conidia clearing process we will be able to bring more understanding to *A. fumigatus* infection cases and possibly aid in curing, perchance even preventing this life-threatening condition in immunocompromised patients.

P.D2.02.11

NKG2D sets activation threshold for NCR1 early in NK cell-development and controls sensitivity of cancer immune-surveillance

*V. Jelenčić[1], M. Lenartić[1], T. Holmes[2], M. Prchal[3], V. Sexl[3], Y. T. Bryceson[4], F. M. Wensveen[1], **B. Polic**[1];*
[1]Faculty of Medicine, University of Rijeka, Rijeka, Croatia, [2]Broegelmann Laboratory, Department of Clinical Sciences, University of Bergen, Bergen, Norway, [3]Institute of Pharmacology and Toxicology, Department for Biomedical Sciences, University of Veterinary Medicine Vienna, Vienna, Austria, [4]Center for Hematology and Regenerative Medicine, Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden.

Introduction: NKG2D and NCR1 (NKp46) are both activating receptors expressed on all NK cells during NK cell development and have important role in the stress-surveillance. 'Stressed' cells up-regulate NKG2D and/or NCR1 ligands which can engage their receptors and activate NK cells. Previously, our group has shown that NKG2D-deficiency affects NK cell development (Zafirova et al. Immunity 2009). Klrk1$^{-/-}$ mice showed an enhanced NK cell-mediated resistance to MCMV infection, while they kept impaired ability to kill NKG2D-expressing tumor targets. **Aim**: Here we investigated molecular mechanism underlaying the NK hyperreactivity and how it influences control of tumors which do not express NKG2D ligands. **Materials and Methods:** In our research we used two tumor models: γ irradiation-induced thymoma and B16 melanoma. We also used different functional assays, flow cytometry and various genetically modified mice to investigate roles of specific receptors and signaling molecules. **Results**: NKG2D-deficiency results in specific NCR1-medicated NK cell hyperreactivity. The hyperreactivity occurs during the NK cell development and is due to the lack of signaling through NKG2D-DAP12 axis. It is correlated with reduced expression of CD3ζ and Zap70. The hyperreactivity results in better control of the investigated tumors and MCMV infection in Klrk1$^{-/-}$ and DAP12$^{-/-}$ mice. **Conclusion**: This research shows for the first time that an activating NK receptor controls activity of another one. Early during NK cell development NKG2D/DAP12 axis sets threshold for NCR1 which leads to NK cell hyperreactivity and better control of MCMV infection and tumors expressing NCR1 ligands.

P.D2.02.12

Peptide-specific recognition of human cytomegalovirus strains controls adaptive natural killer cells

*Q. Hammer[1], **T. Rückert**[1], C. Romagnani[1,2];*
[1]Innate Immunity, German Rheumatism Research Center (DRFZ), Leibniz Association, Berlin, Germany, [2]Charité Universitätsmedizin, Berlin, Germany.

Natural killer (NK) cells are innate lymphocytes that lack antigen-specific rearranged receptors, a hallmark of adaptive lymphocytes. In some people infected with human cytomegalovirus (HCMV), an NK cell subset expressing the activating receptor NKG2C undergoes clonal-like expansion that partially resembles anti-viral adaptive responses. However, the viral ligand that drives the activation and differentiation of adaptive NKG2C$^+$ NK cells has remained unclear. Here we found that adaptive NKG2C$^+$ NK cells differentially recognized distinct HCMV strains encoding variable UL40 peptides that, in combination with pro-inflammatory signals, controlled the population expansion and differentiation of adaptive NKG2C$^+$ NK cells. Thus, we propose that polymorphic HCMV peptides contribute to shaping of the heterogeneity of adaptive NKG2C$^+$ NK cell populations among HCMV-seropositive people.

P.D2.02.13

Neutrophils suppress mucosal associated invariant T cells

***M. Schneider**, J. E. Ussher;*
Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand.

Mucosal associated invariant T (MAIT) cells are abundant innatelike lymphocytes which are rapidly activated in response to bacterial and fungal infections. Upon activation MAIT cells produce cytokines, including INFγ and TNFα, and upregulate cell surface markers CD69 and CD137 (4-1BB). Neutrophils are crucial for the early immune response and have been shown to suppress the activation of innatelike lymphocytes, such as γδ T cells and iNKT cells. Here, we investigated the influence of neutrophils on MAIT cell activation. MAIT cells, monocytes, and neutrophils were isolated from the blood of healthy donors. Cells were stimulated with fixed bacteria, and their activation assessed by flow cytometry. We show that neutrophils supressed the activation of MAIT cells, both in cocultures with monocytes and in PBMCs after stimulation with fixed bacteria. The production of effector cytokines INFγ and TNFα, as well as the upregulation of cell surface markers CD69 and CD137 (4-1BB), was inhibited. Therefore, neutrophils suppress the activation of MAIT cells and may play an important role in the regulation of the innate immune response to extracellular bacteria through the inhibition of innatelike lymphocytes. Investigations into the mechanism of suppression are ongoing with results to be presented at the conference.

P.D2.02.14

***In vivo* depletion of T-bet in intestinal innate lymphoid cells**

***J. Schroeder**[1], J. Lo[1], L. Roberts[1], H. Helmby[2], G. Lord[1];*
[1]King's College London, London, United Kingdom, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom.

Introduction: Innate lymphoid cells (ILCs) have been suggested to play important roles at mucosal surfaces primarily by the expression of subset-specific cytokines regulated by lineage-defining transcription factors. However, due to the lack of appropriate mouse models the functional redundancy of ILC in certain models cannot be excluded. Method: We generated T-bet fl/fl x Cre-Ert2 mice allowing the tamoxifen-induced depletion of T-bet *in vivo*. Breeding pairs were set up in order to generate Cre-Ert2 positive and negative litters. Tamoxifen was administered via the intraperitoneal route on 5 consecutive days. Mice were rested until 3 weeks post the initial injection of tamoxifen. Afterwards cells were harvested without further treatment or upon exposure to dextran sulfate sodium (DSS)-containing drinking water or intestinal infection with *N. brasiliensis* or *H. polygyrus*. Results/ conclusion: Here we show that T-bet is crucially important to maintain NKp46$^+$ NK1.1$^+$ CD127$^+$ ILC in the colonic and small intestinal lamina propria. In contrast to CD127$^+$ ILC, T-bet expression in CD4$^+$ T cells was only partially diminished. Strikingly, upon tamoxifen-induced depletion of T-bet$^+$ CD127$^+$ ILC mice showed significantly less weight loss upon DSS challenge. This observation stands in contrast to models of intestinal infection with *N. brasiliensis* or *H. polygyrus* as depletion of T-bet - expressing CD127$^+$ ILC did not result in accelerated parasite depletion. Furthermore there was minimal evidence of ILC plasticity following temporally defined T-bet deletion. Hence, our novel model of specific depletion of T-bet in CD127$^+$ ILC points to the crucial role of this transcription factor in mucosal inflammation.

P.D2.02.15

Galectin-3 deficiency promotes liver inflammation and facilitates TNF-α-dependent hepatocyte death in MCMV infection

***B. S. Stojanovic**[1], I. Strazic Geljic[2], A. Arsenijevic[1], J. Milovanovic[1], N. Arsenijevic[1], S. Jonjic[1], M. L. Lukic[1], M. Milovanovic[1];*
[1]Faculty of medical science, Kragujevac, Serbia, [2]Faculty of Medicine, Rijeka, Croatia.

Galectin-3 (Gal-3) is a lectin that plays various roles in the pathogenesis of malignant, inflammatory, autoimmune and infectious diseases including liver diseases. In this study, using C57BL/6 mice with target deletion Lgals3 gene (Gal-3-/-) for the first time was demonstrated that the absence of Gal-3 enhanced liver damage in hepatitis induced by intraperitoneal application of murine cytomegalovirus (MCMV). Livers of MCMV infected Gal-3-/- mice contained more inflammatory and necrotic foci, necrotic hepatocytes, and significantly higher level of ALT in the sera compared with the group of C57BL/6 (WT) infected mice, 36 and 72 hours after infection. Significant increase in viral titres was detected in the liver of Gal-3-/- mice compared to WT mice, 72 hours after infection. TNF-α expression was detected in hepatocytes of MCMV infected mice and it was higher in the livers of Gal-3 KO mice compared with the group of WT mice. The number of TNF-α-positive hepatocytes isolated from the livers of infected mice and and concentration of TNF-α liver tissue homogenate was significantly higher in the group of Gal-3 KO mice. TNF-α blockade with monoclonal antibodies before MCMV infection significantly reduced hepatocyte necrosis only in Gal-3 KO mice. MCMV infection increased the expression of Gal-3 on hepatocytes of WT mice. Treatment with Gal-3 inhibitor (TD139) enhanced liver necrosis in WT mice and administration of recombinant Gal-3 reduced inflammation and liver damage in Gal-3 KO mice. This study demonstrated that Gal-3 plays a protective role in hepatitis induced with murine cytomegalovirus infection by reducing TNF-α-induced hepatocyte death.

P.D2.02.16

The role of IFNγ in patients with ulcerative colitis

***A. Valeeva**[1], O. Skorokhodkina[1], R. Abdulkhakov[1], S. Abdulkhakov[2], A. Rizvanov[2];*
[1]Kazan State Medical University, Kazan, Russian Federation, [2]Kazan Federal University, Kazan, Russian Federation.

Ulcerative colitis (UC) is a clinical form of inflammatory bowel diseases (IBD) and considered as a chronic disorder of the gastrointestinal tract characterized by intestinal inflammation and epithelial injury. The pathogenesis of UC remains unclear. Disease has been considered to be associated with a non-conventional Th2 response. Besides the role of Th17 in the pathogenesis of autoimmune inflammation in UC is being discussed. Nowadays results from numerous studies indicate a role for innate lymphoid cells (ILC) in the pathogenesis of chronic intestinal inflammation in IBD.Objective: To analyze the serum levels of IFNγ in patients both in the acute stage and remission of UC. Methods: Forty eight patients in the acute stage and twenty patients in remission of UC were included into the study. Serum cytokine levels were analyzed using multiplex immunoassay. Statistical analysis was performed using STATISTICA 6.0 Software Package. 11 healthy volunteers were included into the control group.Results: Statistically significant increase of IFNγ in serum of patients both in acute stage (176,15 pg/ml [65,15;359,84]) and remission (42,6 pg/ml [29,4;64,45]) compared to controls (16,5 pg/ml [12,3;23,2], p=0,00107; 0,0118) respectively) was revealed. ILC type 1 are defined by production of proinflammatory IFNγ , which is discussed nowadays to play a substantial role in chronic inflammation in IBD. Therefore increase of IFNγ might be a marker for functional overactivity of ILC.Conclusions: Increased levels of IFNγ might suggest overactivity of innate lymphoid cells. Innate lymphoid cells may contribute to chronic immune inflammation in the pathogenesis of ulcerative colitis.

P.D2.02.17

Innate lymphoid cells in paediatric inflammatory bowel disease

***A. Van Acker**[1], E. Kvedaraite[1,2], M. Lourda[1,2], M. Ideström[3], J. Henter[2], M. Svensson[1], J. Mjösberg[1];*
[1]Center for Infectious Medicine, Karolinska Institutet, Stockholm, Sweden, [2]Childhood Cancer Research Unit, Department of Women's and Children's Health, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden, [3]Paediatric Gastroenterology, Hepatology and Nutrition Unit, Department of Women's and Children's Health, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden.

Background: The underlying etiology of paediatric inflammatory bowel disease (PIBD) remains largely unknown, indicating the dire need for more knowledge on the mechanisms driving this disease. Recent publications have highlighted the importance of ILCs in murine and adult IBD development and progression. In this project, we aim to study ILC heterogeneity and function specifically in PIBD. **Methods:** Peripheral blood mononuclear cells (PBMCs) and single-cell suspensions were isolated from blood and colon biopsies, respectively, of 20 PIBD and 5 non-PIBD patients admitted to the Paediatric Gastroenterology, Hepatology and Nutrition Unit at Karolinska University Hospital, Sweden. ILC population frequency and phenotype was examined by 18-colour flow cytometry and correlated to children's IBD physician global assessment (PGA) scores. **Results:** Preliminary results from our flow cytometry data show a statistically significant decrease in the frequency of ILC3 cells ($p<0.05$) and a statistical tendency towards an increase in the frequency of ILC1 ($p=0.06$) in the gut of PIBD as compared with non-PIBD control patients. In PBMC, we detected a slight decrease in the frequency of ILC1 ($p=0.06$). **Conclusions:** Our data suggests a skewing of the ILC balance in the intestinal mucosa of PIBD patients, with an increase of IFN-γ producing ILC1, and a decrease of IL-22 producing ILC3. However, our results are based on a small patient cohort and as yet lack sufficient power for our intended statistical analysis. Extensive further research will now be conducted to continue this analysis as well as examine additional phenotypical and functional differences in ILCs from PIBD patients and non-PIBD controls.

P.D3.01 Novel approaches to vaccinology - Part 1

P.D3.01.01

Investigation of immunogenic properties of Hemolin from *Bombyx mori* as carrier protein: an Immunoinformatic approach.

S. Aathmanathan[1], *V. K. Prajapathi*[2], *M. Krishnan*[1,2];
[1]*Bharathidasan University, Trichy, India,* [2]*Central University of Rajasthan, Ajmer, India.*

Encapsulated bacteria are pathogens which causes disease among elderly, infants and immune-compromised individuals. Since polysaccharides (haptens) are less immunogenic, carrier proteins are conjugated with haptens to elicit a stronger immune response and prolonged T-cell memory. Dendritic cells are the most potent antigen presenting cells (APC's). Toll like receptors (TLR's) present on APC's plays a major role in identification of antigen and activation of cell mediated immunity. Hence TLR agonists are potential immunostimulants. Carrier protein which enhances the cell mediated immunity through TLR activation is a potent candidate for conjugate vaccine. Hemolin,48 kDa protein which is present in major lepidopteran insects, that is similar to mammalian immunoglobulin. It has also a natural affinity towards the bacterial lipopolysaccharides. In the present study, Hemolin was modelled using RaptorX server. The model was validated using RAMPAGE and ProsaWeb servers. LPS of *E.coli*, TLR3 and TLR4 of *Homo sapiens* were docked with Hemolin using Patchdock and interaction was visualized using Chimera 1.11. Docked complexes were subjected to molecular dynamics for 20ns using GROMACS standalone tool. B and T-cell epitopes were identified using tools in IEDB server. IFN-γ activation epitopes were identified using IFN epitope server. The allergenicity was predicted using Allertop and AllerFP servers. This makes hemolin a suitable candidate as a carrier protein in conjugate vaccine because of its high immunogenicity and TLR activation.

P.D3.01.02

In silico designing of a novel multi-epitope peptide vaccine against *Leishmania infantum*: Analysis of its immunogenic potential *in vitro* and *in vivo*

M. Agallou, *E. Athanasiou, M. Margaroni, E. Karagouni;*
Laboratory of Cellular Immunology, Department of Microbiology, Hellenic Pasteur Institute, Athens, Greece.

In the present study, we have designed a multi-epitope peptide vaccine referred as LiChimera, containing several Helper (HTL) and Cytotoxic (CTL) T lymphocyte epitopes obtained from different *Leishmania infantum* proteins through computational vaccinology approaches. The selected epitopes were fused together by using appropriate linkers, while the N-terminal domain of Heparin-Binding Hemagglutinin (HBHA) from *Mycobacterium tuberculosis* was also linked as a TLR4 agonist. LiChimera was effectively expressed in *E. coli* system and its immunogenicity was determined by injecting it intramuscularly alone or in the presence of Addavax - a squalene-based adjuvant - twice at 2 weeks intervals in BALB/c mice.
Results indicated that LiChimera was highly immunogenic and elicited antigen-specific adaptive immune responses, as shown by the high level of serum IgG production and splenocyte proliferation, which were further enhanced in the presence of Addavax. In addition, bone-marrow derived macrophages obtained from mice that received LiChimera and Addavax demonstrated increased leishmanicidal activity confirmed by reduced parasite load in comparison to macrophages obtained from control mice. Overall, we described a new multi-epitope peptide vaccine and its immunogenic properties as a candidate vaccine against leishmaniasis. Further experimentation will be conducted in order to determine its protective efficacy against infectious challenge of *Leishmania*. *This research was made possible through the grant from the Stavros Niarchos Foundation to the Hellenic Pasteur Institute, as part of the Foundation's initiative to support the Greek research center ecosystem.*

P.D3.01.03

Adjuvants enhance induction of germinal center and antibody secreting cellsin spleen of neonatal mice and their persistence in bone marrow

A. A. Aradottir Pind[1,2], *M. Dubik*[1,2], *S. S. Thorsdottir*[1,3], *J. Holmgren*[4], *A. Meinke*[5], *G. Del Giudice*[6], *S. P. Bjarnarson*[1,2], *I. Jonsdottir*[1,2];
[1]*Department of Immunology, Landspitali, the National University Hospital of Iceland, Reykjavík, Iceland,* [2]*Faculty of Medicine, School of Health Sciences, University of Iceland, Reykjavík, Iceland,* [3]*Karolinska Institute, Stockholm, Sweden,* [4]*University of Gothenburg Vaccine Research Institute (GUVAX), Department of Microbiology and Immunology, University of Gothenburg, Gothenburg, Sweden,* [5]*Valneva Austria GmbH, Vienna, Austria,* [6]*GSK Vaccines, Siena, Italy.*

Introduction: Immaturity of the immune system contributes to poor vaccine responses in early life. Germinal center (GC) activation is limited due to poorly developed follicular dendritic cells (FDC), causing generation of few antibody-secreting cells (AbSCs) with limited survival and transient antibody responses. The potential of five adjuvants to overcome limitations of the neonatal immune system to induce more robust and prolonged vaccine responses was explored.
Materials and methods: Neonatal mice were immunized with a pneumococcal conjugate vaccine Pnc1-TT w/wo adjuvants LT-K63, mmCT, MF59, IC31 or alum. Spleen, bone marrow (BM) and blood were collected at various time points after immunization. Spleen sections were stained for FDC maturation and GC activation, vaccine-specific AbSCs were enumerated in spleen and BM with ELISPOT and vaccine-specific serum-antibodies measured with ELISA.
Results: Mice immunized with Pnc1-TT with LT-K63, mmCT, MF59 or IC31 had significantly enhanced maturation of FDCs compared to mice immunized with vaccine alone. LT-K63, MF59 and IC31 significantly enhanced GC formation and mmCT and MF59 significantly enhanced vaccine-specific AbSCs in spleen 14 days after immunization. Neonatal mice immunized with Pnc1-TT with LT-K63, mmCT, MF59 or IC31 had significantly enhanced numbers of vaccine-specific AbSCs in BM 9 weeks after immunization and significantly enhanced vaccine-specific serum antibodies persisting above protective levels against pneumococcal bacteremia and pneumonia.
Conclusion: LT-K63, mmCT, MF59 and IC31 overcame limitations of the neonatal immune system and enhanced both induction and persistence of protective immune response when administered with Pnc1-TT. They are therefore promising candidates for further research on neonatal immune responses.

P.D3.01.04

Immunogenicity of DNA vaccines delivered by patches &lt electroporation in pig skin

C. Bernelin-Cottet[1], *C. Urien*[1], *F. Blanc*[2], *J. Leplat*[2], *J. McCaffrey*[3], *D. Collins*[3], *V. Jakob*[4], *E. Bordet*[1], *C. Barc*[5], *V. Contreras*[6], *N. Bertho*[1], *N. Collin*[3], *C. Barnier-Quer*[4], *A. Moore*[7], *I. Schwartz-Cornil*[1];
[1]*VIM-INRA-Université Paris-Saclay, Jouy-en-Josas, France,* [2]*GABI-INRA-AgroParisTech-Université Paris-Saclay, Jouy-en-Josas, France,* [3]*School of Pharmacy, University College Cork, Xeolas, Cork, Ireland,* [4]*Vaccine Formulation Laboratory, Department of Biochemistry, University of Lausanne, Epalinges, Switzerland,* [5]*INRA, UE1277, Plate-Forme d'Infectiologie Expérimentale, PFIE, Nouzilly, France,* [6]*CEA-Université Paris Sud-INSERM, U1184 « Immunology of viral infections and auto immune diseases », IDMIT department, IBFJ, Fontenay-aux-Roses et Kremlin-Bicêtre, France,* [7]*School of Pharmacy, University College Cork, Xeolas, Cork, France.*

DNA vaccines show suboptimal efficacy in humans and domestic species. The delivery of DNA vaccine is key to improve immunogenicity. As skin is a readily accessible tissue rich in antigen presenting cells and pig skin is a relevant models for humans, we evaluated different modes of DNA delivery in pig skin, i.e. surface electroporation (EP) and dissolvable microneedle patches (DMN), and we assessed the benefit of DNA adsorbtion on cationic poly(lactic-co-glycolic acid) nanoparticles (NP). We used plasmids encoding for luciferase and vaccine plasmid encoding for weak antigens derived from the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), an arterivirus responsible of reproductive disorders in sows and respiratory illnesses in piglets. All methods were successful at inducing luciferase expression in skin. At 24 hours post administration, EP, and not DMN, induced a strong recruitment of granulocytes in skin, with the reduction of conventional dendritic cell subsets (cDC1 and cDC2) and of Langerhans cell, in association to a high production of IL1b and IL8. Substantial T cell responses against the PRRSV antigens were induced upon delivery with EP and DMN. Notably we obtained the broadest IFNg T response against a large panel of PRRSV antigenic regions with DNA adsorbed on NP and delivered by EP. Good systemic and mucosal IgG responses were induced by EP and not by DMN delivery. Altogether, delivery of DNA vaccines with electroporation and patches can be achieved in pig skin with variable degrees of transduction efficacy and local inflammation and can induce immune responses against weak antigens.

P.D3.01.05

Bipolymer based Novel Nanoparticles in Microsphere System as Vaccine Adjuvant

S. Bhargava[1], *V. Bhargava*[2];
[1]*Himalayan University, Kanpur, India,* [2]*GTB Hospital, Kanpur, India.*

Novel strategies are required for the achievement of safe and effective immunization beyond conventional strategies.Frequent booster dosing can be avoided by development of mucosal/adjuvant vaccine delivery system, which can produce both humoral and cell-mediated responses. The work envisaged uses combined hydrophilic(gelatin nanoparticles, GN) with a hydrophobic polymeric system(PLGA microspheres) which creates a biodegradable system for HBsAg delivery. GN & PLGA microspheres were prepared by double emulsification method and composite system by phase separation method.Composites were optimized and characterized *in-vitro* for their shape, size by Scanning & Transmission Electron Microscopy, %antigen entrapment and stability. Fluorescence microscopy was carried out to confirm the uptake. *In-vivo* study comprised of estimation of IgG response in serum and sIgA in various body secretions using specific ELISA. The *in-vitro* studies exhibited an initial burst release from gelatin nanoparticles, degradation of antigen from PLGA microspheres & a continuous release from composite system. This supports the hypothesis to formulate single shot vaccine with such system (to mimic booster dosing). The fluorescence studies showed the selective uptake of composites by NALT. Humoral response generated by single dose of composites was comparative to marketed formulation receiving booster dose. Further, composite system generated effective sIgA antibody which was not elicited by marketed formulation.Thus, it could be concluded from present study that bipolymer based composite system are capable to provide sufficient protein stability and can be a promising candidate for development of single shot vaccine, not only against Hepatitis but against all those diseases that invade host by mucosal surfaces.

P.D3.01.06
Oral combination vaccine against Anthrax & Hepatitis B: Development & Characterization

M. Bhargava[1], *S. Bhargava*[2];
[1]*GTB Hospital, Kanpur, India,* [2]*Himalayan University, Kanpur, India.*

Infections are still leading cause of morbidity and mortality and most of which can be prevented by vaccination. However, there are too many vaccines to be administered, increasing cost of immunization. Combination vaccines can answer these problems by development of single vaccine containing all possible antigens. The goal of present study was to see the effect of 2 antigens when given in combination. Bilosomes can provide needle free, painless approach for immunization. Recombinant hepatitis-B surface antigen(HBsAg) and recombinant protective antigen(rPA) were candidate antigens.
Bilosomes containing rPA and HBsAg were prepared by lipid cast film method. Antigen loaded bilosomes were characterized *in-vitro* for shape, size, antigen entrapment and stability in various body fluids. Fluorescence microscopy was done to confirm the uptake of bilosomes. The *in-vivo*study comprised of immunization of Balb/c mice and estimation of IgG response in serum and sIgA in various body secretions using specific ELISA.
Bilosomes formed were multilamellar and stable in gastric and intestinal fluids. Fluorescence microscopy suggested that bilosomes were taken up by gut associated lymphoid tissues. *In-vivo*data demonstrates that combination produced both systemic as well as mucosal antibody responses upon oral administration at higher dose levels as compared to intramuscular immunization but fail to produce any synergistic effect.
When rPA and HBsAg given in combination, HBsAg(high dose) potentiates the production of anti-rPA antibody. Also they elicited measurable sIgA in mucosal secretions, while alum adsorbed antigens failed to elicit such responses. The combination produced both systemic as well as mucosal antibody responses upon oral administration.

P.D3.01.07
***Mycobacterium indicus pranii (Mw)* in combination with heat induced promastigotes persuade host protection against drug- sensitive and - resistant *Leishmania donovani* infection: Activation of Flt3+ preDC leading to IL-6 producing CD11c+ cDC**

S. Dey[1], *D. Mukherjee*[2], *S. S. Sultana*[2], *S. Mallick*[2], *S. Mandal*[2], *A. Dutta*[2], *P. Patra*[3], *B. Saha*[4], *C. Pal*[5];
[1]*West Bengal State University (work station address) Barasat Govt College (Present address) Dept of Zoology, Kolkata, India,* [2]*West Bengal State University Dept of Zoology, Kolkata, India,* [3]*Canning Subdivisional Hospital, Kolkata, India,* [4]*National Centre for Cell Sciences, Pune, India,* [5]*West Bengal State University Dept of Zoology (Corresponding Author), Kolkata, India.*

Introduction: The major concerns of currently available chemotherapeutics against VL are severe toxicity & resistance. The aim of this work is to design a successful adjuvant in combination with *Mw* & HIP against experimental murine VL.
Methods: 5 Balb/c mice, per group, were administered with s.c. injection of *Mw* (10^8 cells/ kg b.w.) & HIP (50 µg/ kg b.w.) for 7 days after establishing infection. Organs were harvested for flow cytometric & RT-PCR analysis.
Results*: Mw+HIP* effectively reduced the hepatic & splenic parasite burden of infected animals by inducing CD4+IFN-γ+ T cells, along with the upregulation of Th1 promoting cytokines, chemokines & TLR2. We demonstrated that this therapy requires the cooperation of an integrated TLR~Cytokine~Chemokine loop. We established the roles of IL-6 & IL-12p40 as critical cytokines that mediate anti-*Ld* host protection. *Mw+HIP* induced the expansions of CD11c+CD11b+, CD11c+CD8α+ splenic cDCs & $CD11c^{interim}$ B220+PDCA1+ splenic pDCs along with the up regulated expressions of IL-6 & IL-12p40. *Mw+HIP* restored the depleted bone marrow system, *in vivo* & could direct bone marrow CDP cells to preDCs, ultimately repopulating the DCs. It also induced collateral host protective responses by diminishing the CD4+ CD25+ FoxP3+ IL-10+TGF-β+ regulatory T cells, and increasing the CD4+ IL17+ Th17 cells *in vivo*. Interestingly, *Mw+HIP* were found effective against Miltefosine resistant-*L. donovani* (HePC-R) *in vitro & in vivo* as evidenced by the concomitant surge in iNOS level & limited expression of amastigote specific *Ld-kDNA*.
Conclusion: This novel combinational therapy cures murine VL by the pro-inflammatory host protective immune responses.

P.D3.01.08
How to improve diphtheria vaccination for the elderly

M. Grasse*, A. Meryk, C. Miggitsch, B. Grubeck-Loebenstein;*
Institute for Biomedical aging research, University of Innsbruck, Innsbruck, Austria.

We previously demonstrated booster vaccinations with multivalent tetanus/diphtheria vaccines provided long-term protection to tetanus, while long-lasting immunity against diphtheria was insufficient in humans, particularly in the elderly. To investigate the reason for that, we set up a mouse model with different vaccination regimes, consisting of varying numbers of primary and booster vaccinations. Furthermore, we targeted dendritic cells(DCs) by application of GMCSF, and measured humoral & cellular immune responses by ELISA and flow cytometry. For tetanus we can show, that animals who received a primary immunization with three shots of Infanrix® and additionally three booster shots with Boosterix® had the same antibody titers than animals which received only three booster shots with Boosterix®. The diphtheria-specific antibodies were much lower of the mice that received only booster shots, compared to the mice with primary and booster shots. By applying GM-CSF next to the vaccine, young and old mice had significantly better diphtheria-specific antibody responses. GM-CSF treated mice had more diphtheria-specific CD4+ T-cells producing IL-2, IL-6 and TNF-a. GM-CSF was leading to a higher number of DCs at the injection-site 24h after vaccination and also to more splenic DCs with upregulated MHC-II expression. Our findings demonstrate, that the imbalanced level of protection against tetanus and diphtheria provided by multivalent tetanus/diphtheria vaccines is most likely due to the vaccine composition and not because of the vaccination regimes. Moreover, targeting DCs with GM-CSF improves the diphtheria-specific immune response and this might be a useful strategy to improve the vaccination situation for the elderly.

P.D3.01.09
Novel RNA adjuvant transcribed from viral internal ribosome entry site improves vaccine efficacy enabling immune-stimulatory capacities

H. Kwak*, H. Ko, H. Park, H. Park, R. Kim, J. Nam;*
Catholic University of Korea, Seoul, Korea, Republic of.

Most of the vaccines use aluminum compounds (alum) as an adjuvant to increase adaptive immune response to antigens. However, alum-based adjuvant strongly induce Th2 immune response rather than Th1 response. Here, we developed a novel single-stranded RNA adjuvant to induce balanced Th1 and Th2 responses. The plasmid containing the gene of interest under the internal ribosome entry site of the cricket paralysis virus intergenic region was transcribed by T7 RNA polymerase. The RNA adjuvant treated bone marrow derived dendritic cells (BMDCs) showed higher expression of activation marker. Furthermore, the RNA adjuvant stimulated immune-related genes in BMDCs. In addition, the RNA adjuvant injected mice showed higher antigen specific IgG1 (indicative of Th2 response) and IgG2a (indicative of Th1 response), as well as neutralizing antibody against antigen, compared to those in alum adjuvant injected group. Furthermore, the RNA adjuvant injected group showed higher antigen specific T cells secreting IFN-γ than alum adjuvant injected group. Taken together, the RNA adjuvant can increase humoral and cellular immune responses to vaccine antigen and act as a promising immunoenhancer.

P.D3.01.10
Human milk oligosaccharide improves innate and adaptive immunity and alters gut microbiota composition in an influenza-specific murine vaccination model

X. Ling*;*
Utrecht Institute for Pharmaceutical Sciences, Utrecht, Netherlands.

Human milk is uniquely suited to provide optimal nutrition and immune protection to infants. 2'-Fucosyllactose (2'FL) is one of the most predominant oligosaccharides present in and associated with the immune benefits of human milk. The effect of 2'FL on vaccination responsiveness and mechanisms involved was determined.
A dose range of 0.25-5% (w/w) dietary 2'FL was provided to a murine influenza vaccination model. Vaccine-specific delayed-type hypersensitivity (DTH), antigen-specific antibody levels in serum, and immune cell populations within several organs were evaluated. The effects of 2'FL on vaccine-specific T-cell proliferation and cytokine secretions, and the direct immunomodulatory effects of 2'FL were assessed using *ex vivo* bone marrow-derived dendritic cells (BMDCs) T-cell co-culture. Finally, the impact of 2'FL on the gut microbiota composition was evaluated.
Dietary 2'FL effectively enhanced vaccine specific DTH responses and serum vaccine-specific IgG1 and IgG2a levels in a dose-dependent manner. Consistently, higher frequency of B-cells was detected in mice receiving 2'FL. Moreover, proliferation of vaccine-specific CD4+ and CD8+ T-cells, and IFN-γ production after *ex vivo* restimulation were significantly increased in spleen cells of mice receiving 2'FL, which were in line with changes detected within DC populations. Direct effect of 2'FL on the maturation status and antigen presenting capacity of BMDCs was confirmed *in vitro*. And the microbiota profile was significantly changed by 2'FL.
Dietary intervention with 2'FL improves both humoral and cellular immune responses to vaccination in mice, which might be attributed both direct immunomodulatory effects and microbiota modification of 2'FL.

P.D3.01.11

Inflammatory monocytes differentiate into mature antigen presenting cells *ex vivo* by oligomannose-coated liposomes

Y. Matsuoka[1], *Y. Kawauchi*[1], *Y. Kuroda*[1], *K. Kawauchi*[2], *A. Takiyama*[1], *N. Kojima*[1];
[1]*Tokai university, Hiratsuka, Japan,* [2]*Tokyo Women's Medical University, Tokyo, Japan.*

Oligomannose-coated liposomes (OMLs), in which the antigens are entrapped, have been shown to serve as effective antigen delivery vehicles and as a novel adjuvant to induce antigen-specific cellular immune response. Here, we demonstrate that inflammatory monocytes in PBMC can differentiate into mature professional antigen presenting cells *ex vivo* in response to uptake of OMLs.
When PBMC from C57BL/6 was co-cultured with OMLs in the presence mouse serum, OMLs rapidly was incorporated to $CD11b^+/Ly6C^+$ mouse inflammatory monocyte. In addition CD86, CCR7, CD83, and MHC class II significantly was upregulated within 24 h after OML uptake. OVA-encasing OML-ingesting monocytes can activate $CD8^+$ T cells from OT-1 mice, suggesting that antigens encapsulated in OMLs were cross-presented in inflammatory monocytes. Adoptive transfer of the monocytes that engulf OVA-encasing OMLs led to induction of an antigen-specific Th1 immune response in mice. We also observed that OMLs was preferentially incorporated into human $CD14^+$ monocyte in vitro. In response to in vitro OML uptake, CD14+ monocytes matured accompanied with enhanced expression of HLA-DR and CD86, and with secretion of IL-12. Furthermore, CD209, CD123, and CD169 were expressed on $CD14^+$ monocytes in response to OML treatment. Taken together, mature antigen presenting cells, which can activate CD8 T cells, is generated from inflammatory monocytes in peripheral blood by *ex vivo* treatment of the cells with OMLs without any additional stimuli.

P.D3.01.13

Characterization of a nucleoside-modified mRNA vaccine against HIV-1

N. Pardi[1], *C. C. LaBranche*[2], *P. Polacino*[3], *D. C. Montefiori*[2], *S. Hu*[3,4], *D. Weissman*[1];
[1]*University of Pennsylvania, Department of Medicine, Philadelphia, United States,* [2]*Duke University Medical Center, Department of Surgery, Durham, United States,* [3]*Washington National Primate Research Center, University of Washington, Seattle, United States,* [4]*Department of Pharmaceutics, University of Washington, Seattle, United States.*

Introduction: Great progress has been made in understanding the mechanisms of HIV-1 infection, but no effective vaccine worthy of clinical development has been developed to date. In recent years, numerous studies have demonstrated that mRNA-based vaccines could elicit potent immune responses against various infectious pathogens. We have generated a vaccine platform where nucleoside-modified mRNAs were encapsulated into lipid nanoparticles (LNPs), which have recently proved to be safe and efficient tools for *in vivo* nucleic acid delivery. Materials and Methods: Rhesus macaques were intradermally immunized five times with clade C HIV-1 1086C envelope (Env) gp160-encoding nucleoside-modified mRNA-LNPs and antibody responses were assessed by ELISA and virus neutralization assays (standard TZM-bl luciferase reporter system).
Results: Animals generated anti-gp120-specific antibodies, as measured by ELISA. Potent neutralization activity against a highly neutralization sensitive clade C Tier 1 virus (MW965.26) was detected in all animals after four immunizations. Importantly, 3 out of 6 animals developed neutralizing antibodies against the autologous Tier-2 virus (Ce1086_B2). Interestingly, one animal generated antibodies with low neutralizing activity against heterologous clade C Tier-2 strains (25710-2.43 and Ce1176_A3).
Conclusions: Our results demonstrate that antigen-encoding nucleoside-modified mRNA complexed in LNPs induces Tier-2 neutralizing antibody responses in non-human primates. Further characterization of anti-HIV immune responses in this study is underway.
This work was supported by R01-AI124429 and R01-AI084860.

P.D3.01.14

Zika virus DNA vaccine construction and evaluation of its immunogenicity in mice

E. Prompetchara[1], *P. Bamrungchaokasem*[1], *B. Sukchawalit*[1], *C. Ketloy*[2], *K. Ruxrungtham*[2];
[1]*Department of Biochemistry and Microbiology and Vaccines and Therapeutic Protein Research Group (STAR), Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand,* [2]*Chula Vaccine Research Center (ChulaVRC), Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.*

Major ZIKV outbreak occurred during 2015-2016 worldwide. Although most of the ZIKV infections are asymptomatic, unfortunately, there are several reports on severe complications such as microcephaly in newborns and Guillain Barre Syndrome in adults. Currently, either specific treatment or approved ZIKV preventive vaccine are not available. This research, therefore, emphasizes on the study and development of the DNA vaccine against ZIKV as it is easy and fast to produce, thermostable and efficient in immune induction especially when administered with the effective delivery systems.
ZIKV pre-membrane and envelope prM/E consensus proteins were analyzed from ZIKV isolates deposited in the GenBank database. The humanized codons of the consensus ZIKV *prM/E* were subcloned into the mammalian expression vector (pCMVkan). The protein expression in mammalian (Vero) cell transformed with pCMVkan encoding ZIKV *prM/E* was investigated. ZIKV envelope proteins weredetected extra- and intracellularly when analyzed by western blot and indirect immunofluorescence, respectively. The vaccine was then immunized 3 doses, two-week interval in ICR using intramuscular electroporation as a delivery method. The results demonstrated that neutralizing antibodies (NAb) against ZIKV were detected since week 4 (Geometric mean titer, GMT = 10). NAb levels gradually increased at week 6 and week 8 (GMT = 40 and 80, respectively) andare significantly different from those inthe control group (p<0.05). Collectively, this vaccine prototype is immunogenic in mice and warrant further studies foruse as an effective ZIKV vaccine.

P.D3.01.15

Humoral and cellular immune response induced by novel liposomal formulations using aminoacidic amphiphiles and CpG-ODN as immunostimulants

I. G. Reidel[1,2,3], *L. Grippo*[1,4], *L. Bartalini*[1,4], *R. C. Russi*[1,2], *J. C. Lecron*[5,6], *F. Morel*[5], *M. I. García*[1], *D. M. Müller*[4], *C. Veaute*[1], *J. F. Jégou*[5];
[1]*Laboratorio de Inmunología Experimental, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina,* [2]*Consejo Nacional de Investigaciones Científicas y Técnicas, Capital Federal, Argentina,* [3]*Laboratoire Inflammation, Tissus Epithéliaux et Cytokines (LITEC), Université de Poitiers, Poitiers, France,* [4]*Laboratorio de Química Aplicada (LAQUIMAP), Dto. Química Orgánica, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina,* [5]*Laboratoire Inflammation, Tissus Epithéliaux et Cytokines (LITEC), EA 4331, Université de Poitiers, Poitiers, France,* [6]*CHU de Poitiers, Poitiers, France.*

Introduction: Liposomes are vaccine adjuvant systems able to transport hydro- and liposoluble molecules, allowing the co-incorporation of antigen and different immunostimulants. Our aim was to evaluate the adjuvanticity of a cationic liposomal formulation (Lip) with the addition of a CpG oligodeoxynucleotide (CpG-ODN) and/or an aminoacidic amphiphile (Gem) as immunostimulants, in the immunization against recombinant clumping factor of *Staphylococcus aureus* (rClf).
Materials and Methods: Balb/c mice were immunized with: Lip+CpG-ODN+rClf, Lip+Gem+rClf, or Lip+Gem+CpG-ODN+rClf. Mice received three intradermal doses and were sacrificed three days after the last injection. Anti-rClf IgG was evaluated by indirect ELISA in plasma. Lymph nodes cells were analysed by flow cytometry to assess IL-4, IL-17 and IFN-γ production in CD4+ and CD8+ T cells.
Results: Lip+CpG-ODN+rClf, Lip+Gem+rClf and Lip+Gem+CpG-ODN+rClf all led to high IgG levels. Formulations containing Gem or CpG-ODN increased the number of CD4+ lymphocytes. Lip+Gem+CpG-ODN+rClf significantly enhanced CD4+ cells compared to Lip+CpG-ODN+rClf. A similar trend was found for CD8+ lymphocytes. Gem and CpG-ODN were able to induce the production of IL-4 and IFN-γ by CD4+ cells and IFN-γ by CD8+ cells. Only Gem increased the production of IL-17 by CD4+ cells. Lip+Gem+CpG-ODN+rClf induced the highest number of CD4+ and CD8+ cells producing the three cytokines compared to Lip+CpG-ODN+rClf.
Conclusions: Both Gem and CpG-ODN act as immunostimulants when introduced in liposomes, but their combination enhances the stimulant effect. It should be noted the cytotoxic cells stimulation by the Lip+Gem+CpG-ODN+rClf formulation, considering the impact of this profile in the design of viral or cancer preventive vaccines.

P.D3.01.16

Immunogenic properties of single component adjuvants for vaccine formulations

L. Rossmann[1], *A. Ulrich*[1], *K. Lindt*[1], *S. Shorte*[2], *M. Hasan*[2], *B. David-Watine*[2], *M. Bastian*[3], *G. van Zandbergen*[1,4];
[1]*Paul-Ehrlich-Institut, Langen, Germany,* [2]*Institut Pasteur, Paris, France,* [3]*Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany,* [4]*University Medical Center, Mainz, Germany.*

An appropriate adjuvant is of pivotal importance for vaccine efficacy and thus should be selected on the basis of the adjuvant's immunogenic modes of action. We hypothesize that adjuvant properties can be correlated to specific innate cellular responses influencing the resulting adaptive immune response.
Here, we aim to investigate the immunogenic properties of ten different single component adjuvants *in vitro*, comprising TLR agonists, aluminium salts, oil-in-water emulsions and saponins. We focus on side-by-side comparison of the adjuvants using human primary immune cells of 30 donors which are evenly distributed in sex and age.
In a first step, all adjuvants were tested for their pyrogenicity using an *in vitro* monocyte activation test. Except for MPLA, all tested adjuvants were non-pyrogenic.
The capacity of the adjuvants to induce lymphocyte proliferation was examined in a co-culture based assay composed of human monocyte-derived dendritic cells and autologous peripheral blood lymphocytes (PBLs). We observed that Pam3CSK4, Gardiquimod, R848, synthetic MPLA, natural-derived MPLA and TDB induced antigen-independent proliferation of PBLs to varying degrees, with R848 being the strongest stimulator. More detailed examination of B-, NK-, NKT-, $CD4^+$ and $CD8^+$ T cells within the proliferated PBL population revealed that each adjuvant promoted the proliferation of different cell types. An ongoing Luminex multiplex analysis will uncover the cytokine profile within the co-culture in the first 24h of adjuvant stimulation.
In summary, this work will help to identify immunological properties of adjuvants and will facilitate the rational design of adjuvant-based vaccines.

P.D3.01.17

Development and immunogenicity in mice of a novel chimeric Virus Like Particles (cVLPs) of MERS-CoV

***W. TAN**, Y. Deng, J. Lan;*
National Institute for Viral Disease Control and Prevention, China CDC, BEIJING, China.

The newly found Middle East respiratory syndrome coronavirus (MERS-CoV) was belonged to the coronavirus genus. It resulted a very high case-fatality rate. While there is no specific prophylactic or therapeutic measures to control its spread. In this study we aimed to acquire a chimeric Virus Like Particles (cVLPs) of MERS-CoV in baculovirus expression system by co-expression of M1 and S. Among them, M1 ,which come from the H5N1 ,was the core of the cVLPs and S which come from the MERS-CoV existed the surface of the cVLPs. The results by the electron microscope showed the spheroid cVLPs with a diameter of 80 to 100 nm, which was similar to the MERS-CoV. It was enveloped and looked like a crown. We immunized intramuscularly the mice with the acquired cVLPs and detected the IgG and neutralizing antibodies in the serm of the immunized mice. Above all, we acquired the immunogenic cVLPs of MERS-CoV by co-expression of M1 (H5N1) and S(MERS-CoV) in a baculovirus expression system.Next, the cVLPs will be developed as an effective vaccine against the MERS-CoV infection.

P.D3.01.18

Proteomic analysis of cholera toxin adjuvant-stimulated human monocytes identifies Thrombospondin 1 and Integrin beta 1 as strongly upregulated molecules involved in the adjuvant action

***M. Terrinoni**[1], J. Holmgren[1], M. Lebens[1], M. Larena[1,2];*
[1]Microbiology and Immunology, Biomedicine, Gothenburg, Sweden, [2]Organismal Biology, Uppsala, Sweden.

Background. Cholera Toxin (CT) and its non-toxic derivatives, mmCT and dmLT, have been shown to be potent adjuvants for mucosally administered vaccines. Their adjuvant activity is mediated via antigen presenting cells (APC), and involves cAMP/proteinkinase A (PKA) pathway and inflammasome-dependent IL1β signaling. We extended our investigation to further elucidate the molecular signal transduction mechanisms activated in APCs by this group of adjuvants.
Methods. First, we employed high-throughput proteomic analysis to investigate differential expression of proteins in CT-treated human monocytes at various time points. Next, bioinformatics analyses allowed us to identify protein abundance and major signaling pathways involved. The expression of selected protein after specific treatments was confirmed by *in vitro* assays. Th17responses, measured by ELISA, were evaluated following co-culture of adjuvants-treated human monocytes and dendritic cells with purified CD4+Tcells. Lastly, siRNAs were used to investigate the effect of knocking down expression of selected genes on adjuvant-treated human monocytes.
Results. We report activation of three main biological pathways among upregulated proteins: cellular organization, metabolism, and immune response. Specifically, amongst proteins classified under immune response category, we note a strong upregulation of thrombospondin-1(THBS1) and integrin beta-1(ITGB1). Th17responses were significantly reduced when THSB1 and ITGB1 in APCs were blocked by siRNAtreatment. Furthermore, blocking of cAMP production or inhibition of PKA activity in APCs were found to abrogate the increased expression of THSB1 and ITGB1 along with the Th17 promoting activity induced by the adjuvants.
Conclusions. Our data demonstrate the importance of THSB1 and ITGB1 in the cAMP/PKA-dependent adjuvant action of CT/mmCT/dmLT on APCs.

P.D3.01.19

Live attenuated influenza vaccines are superior in evoking cross-reactive T cell responses against H7N9 in ferrets compared to other traditional vaccines

***K. van de Ven**, H. van Dijken, F. de Heij, J. de Jonge;*
National Institute of Public Health and the Environment, Bilthoven, Netherlands.

Traditional influenza vaccines primarily invoke a humoral immune response against the viral HA and NA proteins. However, as most elicited antibodies do not possess broad cross-reacting properties, immune escape by the virus is a serious problem. T-cell responses against conserved epitopes of internal influenza proteins could potentially offer protection against a broader spectrum of influenza viruses, including viruses that have the potential to cause pandemics. Current vaccines and adjuvanted formulations hereof, have extensively been researched in mice for their capacity of inducing cross-reactive cellular responses. However, since the ferret is a more suitable model for investigation of vaccine efficacy and more tools are becoming available for analysis of cellular responses, we wondered how the cellular immune response differed between traditional vaccine formulations in ferrets. In order to test this, we thawed PBMCs and spleen lymphocytes from previously vaccinated and challenged ferrets and performed ELISPOTs with influenza NP and M1 peptides and H7N9 viruses. Results indicate that a strong cross-reactive T cell response was evoked by the live attenuated influenza vaccine (LAIV), and to some extend by whole inactivated virus (WIV). The T cell response was marginal in animals vaccinated with split-vaccine. These results argue for the use of influenza vaccine formulations that also evoke cellular immunity in order to offer protection against the continuously antigenic-drifting and potential pandemic influenza viruses.

P.D3.01.20

Induction of virus-specific T cell responses via R848 functionalised calcium phosphate nanoparticles

***C. Wenzek**[1], A. Westendorf[1], J. Buer[1], S. Kollenda[2], M. Epple[2], T. Knuschke[1];*
[1]University Hospital Essen, Essen, Germany, [2]University Duisburg-Essen, Essen, Germany.

Calcium phosphate (CaP) nanoparticles are new promising agents for vaccination as they promote virus-specific immune responses which can protect the organism against infection. The aim of this study was to assess the immunomodulatory effects of a new type of CaP nanoparticles, which are functionalised with toll-like receptor 7 ligand resiquimod (R848), viral antigens and the polymer polyethylenimine (PEI), compared to previously used CpG nanoparticles to induce and reactivate Friend retrovirus (FV) specific T cell responses.
Stimulation of immune cells revealed that R848 loaded CaP nanoparticles are able to effectively activate dendritic cell (DC) subsets *in vitro* and *in vivo*, which in turn triggered effector T cell responses. Here, R848 functionalised nanoparticles were most efficient among the tested particles to induce virus-specific $CD8^+$ T cell responses whereas the induction of $CD4^+$ T cells was impaired. The cause might be a negative effect of PEI on MHC II antigen processing. Despite the activation of innate and adaptive immune cells, immunisation of mice with R848 and viral peptide functionalised CaP nanoparticles provided minor protective immunity against acute and chronic retroviral infection as they display inferior $CD4^+$ and $CD8^+$ T cell responses in comparison with CpG functionalised nanoparticles. Therefore, this study defines the potential advantages of R848 functionalised CaP nanoparticles to induce virus-specific $CD8^+$ T cell responses and illustrates the necessity for $CD4^+$ T cell induction for a protective immunity.

P.D3.01.21

Similar antibody responses elicited in SHIV infected and HIV-1 envelope glycoprotein immunized non-human primates

***J. van Schooten**[1], M. van Haaren[1], C. Cottrell[2], D. Sok[2], D. R. Burton[2], A. Ward[2], G. Shaw[3], R. Sanders[1], M. J. van Gils[1];*
[1]Academic Medical Center, Amsterdam, Netherlands, [2]The Scripps Research Institute, San Diego, United States, [3]Perelman School of Medicine, Philadelphia, United States.

The human immunodeficiency virus (HIV-1) remains a global health threat and the development of an effective vaccine is highly desirable to eradicate the HIV/AIDS pandemic completely. Experimental vaccines against HIV-1 have not yet been able to induce protective immune responses. Recently, soluble native-like HIV-1 envelope glycoproteins, termed BG505 SOSIP.664 trimers, were developed which induced neutralizing serum responses against the homologous virus in both non-human primates (NHP) and rabbits. However, it is still unclear whether these native-like trimers elicit similar responses compared to natural infection. Therefore, NHP were immunized with BG505 SOSIP.664 trimers or infected with the homologous BG505 SHIV. Immunogen-specific IgG+ B cells from BG505 SOSIP immunized and BG505 SHIV infected NHP were single cell sorted to obtain monoclonal antibodies (mAbs). The viral population from the BG505 SHIV infected NHP was sequenced over time to study HIV-1 escape from antibody responses. Both neutralizing and non-neutralizing mAbs were isolated and these mAbs targeted distinct epitopes on the HIV-1 trimer, such as the fusion peptide and strain-specific holes in the glycan shield of BG505 SOSIP trimer. mAbs elicited by the BG505 SOSIP trimer showed similar phenotypic characteristics compared to mAbs isolated from the BG505 SHIV infected NHP, however mAbs from the SHIV infected NHP neutralized the homologous virus more frequently and substantial more B-cell clonal expansion was observed during SHIV infection. In conclusion, the results suggest that immunizing with the BG505 SOSIP trimer mimics infection correctly by the induction of mAbs with similar target epitopes. The knowledge obtained in this study will help design immunization strategies to broaden the response after vaccination.

P.D3.01.22

Antibodies from vaccinated rabbits reveal an immunodominant epitope at the bottom of soluble HIV-1 envelope trimers

***M. M. van haaren**[1], C. Cottrell[2], L. E. McCoy[3], P. van der Woude[1], A. Yasmeen[3], S. W. de Taeye[1], A. Torrents de la pena[1], P. Klasse[4], D. R. Burton[2], A. B. Ward[2], R. W. Sanders[1], M. J. van Gils[1];*
[1]Amsterdam university medical centers, Amsterdam, Netherlands, [2]The Scripps Research Institute, San Diego, United States, [3]Weill Medical College of Cornell University, New york, United States, [4]Weill Medical College of Cornell University, New York, United States.

High viral diversity of HIV-1 worldwide requires the development of broadly neutralizing antibodies (bNAbs) through vaccination. Although current vaccine candidates elicit neutralizing antibodies, only limited neutralization coverage is observed. Characterizing antibody responses elicited by current immunogens should provide insight on which epitopes are targeted and what causes the limited breadth. Rabbits were immunized with soluble antigenic mimics of a subtype B HIV-1 envelope glycoprotein (AMC008 Env trimer). Monoclonal antibodies (mAbs) were isolated by single-cell sorting of B cells and subsequent amplification and cloning of the heavy and light chain variable regions of the antibody. We isolated 21 AMC008-specific mAbs, four of which could cross-bind various subtypes.

The complementary determining region three (CDR3) of the light chain of these mAbs were longer than average in rabbits, resembling characteristics of previously isolated bNAbs. Negative stain-electron microscopy and ELISA revealed an epitope at the bottom of the Env trimer targeted by the majority of the isolated mAbs, indicating the immunodominant site of the AMC008 Env trimer. However, the observed approach angle of these mAbs would lead to a clash of the Fc portion with the viral membrane. Nevertheless, homologous AMC008 neutralization, and neutralization of another subtype B virus was observed for four antibodies, potentially achieved by destabilization of the Env trimer on the viral surface. In conclusion, mAbs elicited by AMC008 Env trimer immunizations target the immunodominant bottom of the Env trimer. Viral variability and the suboptimal angle of approach provide an insight in the poor neutralization breadth and should guide future vaccine design.

P.D3.02 Novel approaches to vaccinology - Part 2

P.D3.02.01

Riboflavin supplementation enhances antigen specific humoral and cellular immune responses to oral vaccines

A. S. Albutti[1,2], *C. McEntee*[3], *S. Longet*[1], *A. Svennerholm*[4], *J. Holmgren*[4], *E. Lavelle*[1];
[1]*University of Dublin, Trinity college, Dublin, Ireland,* [2]*Qassim University, Al Qassim, Saudi Arabia,* [3]*University of Manchester, Manchester, United Kingdom,* [4]*University of Gothenburg, Gothenburg, Sweden.*

In contrast to injectable vaccines, effective mucosal vaccines can provide protective local and systemic immunity. Oral vaccination in particular has the potential to offer a safer and more efficacious approach than injection-based approaches, especially in developing countries. Nevertheless, in general, antigen delivery via the oral route triggers weak immune responses or immunological tolerance. The effectiveness of oral vaccination can be improved by co-administering adjuvants. However, a major challenge is the absence of potent and safe oral adjuvants for clinical application. A second obstacle is that responses to oral vaccines vary between developed Western countries and developing countries where they are often less effective. This may be attributed in part to nutritional deficiencies including a limited intake of factors including vitamins, impacting on the microbiome and the generation of specific metabolites. Here, the immunomodulatory potential of oral supplementation with riboflavin (Vitamin B2) was investigated. It was found that oral delivery of the vitamin enhanced antigen-specific Th1 and Th17 responses in draining lymph nodes in addition to antigen specific antibody responses in mice orally vaccinated with the heat killed oral vaccines against enteric bacterial infections. Supplementation with riboflavin prior to and during vaccination with oral cholera vaccine enhanced antigen-specific intestinal and serum humoral immunity. These findings suggest the potential of riboflavin supplementation to enhance the capacity of oral vaccines to trigger mucosal and systemic immunity.

P.D3.02.02

Sensitivity of the bovine BCG challenge model

L. Biffar[1,2], *M. Vordermeier*[2], *H. McShane*[1], *B. Villarreal-Ramos*[2];
[1]*University of Oxford, Oxford, United Kingdom,* [2]*Animal and Plant Health Agency, Weybridge, United Kingdom.*

Bovine tuberculosis is a zoonotic disease affecting cattle and causing economic loss to farmers worldwide. Protection induced, by the currently only experimentally used BCG vaccine, varies between 0 to 80%. Lacking a correlate of protection every new vaccine candidate has to be tested in lengthy and expensive *M. bovis* challenge experiments. Methods that would allow for a faster and cheaper pre-selection of vaccine candidates and additionally improve welfare of experimental animals are needed. We have previously developed a bovine BCG challenge model in which animals are challenged intranodally with a non-pathogenic BCG strain. Improved protection results in reduced bacterial burden in the injected lymph nodes of protected cows compared to unprotected animals three weeks after challenge. The aim of this work was to analyse the sensitivity of the BCG challenge model and thus its ability to differentiate between different vaccine regimens resulting in varying degrees of protection. In order to do so we compared vaccination with BCG only to a heterologous Adenovirus Antigen 85A prime boost vaccine regimen which has previously been demonstrated to improve protection over BCG alone in pathogenic *M. bovis* challenge experiments. We show, that the BCG challenge model is able to measure improved protection of both vaccine regimens over unvaccinated control animals. However, the model is currently not sensitive enough to distinguish between the two different vaccine regimens. Nevertheless, it allows us to determine the ability of new vaccine candidates to induce protection at least as good as BCG.

P.D3.02.03

Presentation of Hepatitis B virus antigens on human dendritic cells; hunting for novel immune therapy targets by Mass spectrometry

R. Bouzid[1], *K. Bezstarosti*[2], *M. de Beijer*[1], *P. Biesta*[1], *A. Woltman*[1], *J. Demmers*[2], *S. Buschow*[1];
[1]*Erasmus MC Dept. of Gastroenterology and Hepatology, Rotterdam, Netherlands,* [2]*Erasmus MC Dept. of Biochemistry, Rotterdam, Netherlands.*

Approximately 350 million individuals worldwide have a chronic hepatitis B infection and as a consequence are at risk for developing hepatocellular carcinoma (HCC). In addition, 50% of all HCC cases arise in individuals infected with HBV, highlighting the contribution of chronic HBV infection to the high prevalence of HCC. Conventional therapies are not curative, are expensive and need to be taken life-long. We aim to design a therapeutic vaccine for the treatment of chronic HBV, that should boost or initiate immunological responses to critical Human leukocyte antigen (HLA) epitopes. To identify target epitopes we here loaded human monocyte derived dendritic cells with HBV antigens from different sources, isolated HLA I complexes and performed sensitive Mass spectrometry on eluted HLA-peptides. We thereby identify therapeutically relevant HBV-epitopes presented on professional antigen presentation cells. Currently we are expanding our methodology to HLA II and primary DCs.

P.D3.02.04

Mixed mucosal-parenteral immunizations with the broadly conserved pathogenic *Escherichia coli* antigen SslE induce a robust mucosal and systemic immunity without affecting the murine intestinal microbiota

I. Naili[1,2], *J. Vinot*[1], *B. C. Baudner*[1], *A. Bernalier-Donadille*[2], *M. Pizza*[1], *M. Desvaux*[2], *G. Jubelin*[2], *U. D'Oro*[1], ***C. Buonsanti***[1];
[1]*GSK, Siena, Italy,* [2]*Université Clermont Auvergne, INRA, Clermont-Ferrand, France.*

E. coli can cause a vast range of intestinal (InPEC) and extraintestinal (ExPEC) diseases but only a very limited number of antibiotics still remains effective against this pathogen. A broad spectrum *E. coli* vaccine could be a promising alternative to prevent the burden of such diseases, while offering the potential for covering against several InPEC and ExPEC at once. SslE, Secreted and Surface-associated Lipoprotein of *E. coli*, is a widely distributed protein among InPEC and ExPEC. SslE functions *ex vivo* as a mucinase capable of degrading mucins and reaching the surface of mucus-producing epithelial cells. SslE was identified by reverse vaccinology as a protective vaccine candidate against an ExPEC murine model of sepsis, and further shown to be cross-effective against other ExPEC and InPEC models of infection. In this study, we aimed to gain insight into the immune response to antigen SslE and identify an immunization strategy suited to generate robust mucosal and systemic immune responses. We showed, by analyzing T-cell and antibodies responses, that mice immunized with SslE via an intranasal prime followed by two intramuscular boosts developed an enhanced overall immune response compared to either intranasal-only or intramuscular-only protocols. Importantly, we also report that this regimen of immunization did not impact the richness of the murine gut microbiota. Mice had a comparable cecal microbial composition, whether immunized with SslE or PBS. Collectively, our findings further support the use of SslE in future vaccination strategies to effectively target both InPEC and ExPEC while not perturbing the resident gut microbiota

P.D3.02.05

Intranasal delivery of inactivated enterovirus D68 induces robust virus-specific humoral and cellular responses and confers protection against lethal viral challenge in mice

C. Chin[1], *Y. Lin*[2], *P. Cheng*[2], *B. Chiang*[1,2,3];
[1]*Graduate Institute of Immunology, National Taiwan University, Taipei, Taiwan,* [2]*Department of Medical Research, National Taiwan University Hospital, Taipei, Taiwan,* [3]*Graduate Institute of Clinical Medicine College of Medicine, National Taiwan University, Taipei, Taiwan.*

Introduction: Enterovirus D68 (EV-D68) has been recognized as a significant respiratory pathogen in children since its infections have remarkably increased worldwide during the past decade. In addition to severe respiratory tract infection, EV-D68 has been reported to associate with acute flaccid myelitis (AFM) in recent years. However, neither effective vaccines nor antiviral drugs are currently available to prevent its infections. Given that its infections have been demonstrated to depend on mucosal invasion, we sought to develop a potential mucosal vaccine against EV-D68.
Materials and Methods: C57BL/6 mice were intranasally immunized with the inactivated virus, and both humoral and cellular immune responses were assessed after the immunization. Sera collected from the vaccinated mice were further used in neutralization tests and passive protection assays.
Results: We found enhanced EV-D68-specific responses in C57BL/6 mice after the intranasal challenge, including the elevated antibody titers in nasal wash, saliva, bronchoalveolar fluid, feces, and sera, as well as the increased proliferative and IgG-producing ability of the restimulated splenocytes. We also confirmed the sera to have neutralizing and cross-neutralizing capability in human Rhabdomyosarcoma (RD) cells. Furthermore, we found that anti-EV-D68 sera transfer before the intracerebral challenge lowered the incidence of paralysis and prolonged the survival of neonatal Institute of Cancer Research (ICR) mice. Tissue load analysis showed decreased viral levels in the spinal cord and limb muscles of the sera recipients, and the histopathologic examination revealed reduced necrosis of hindlimb muscles of the recipients.
Conclusion: Our data demonstrated the proof-of-concept for effective intranasal vaccination with inactivated EV-D68.

P.D3.02.06

Modulation of primary immune response by different adjuvants to design heterologous prime-boost combinations

A. Ciabattini[1], *E. Pettini*[1], *F. Fiorino*[1], *S. Lucchesi*[1], *G. Pastore*[1], *F. Santoro*[1], *P. Andersen*[2], *G. Pozzi*[1], *D. Medaglini*[1];
[1]*University of Siena, Siena, Italy,* [2]*Statens Serum Institute, Copenhagen, Denmark.*

Adjuvants contribute to enhancing and shaping the vaccine immune response through different modes of action. Here, we profiled early biomarkers of adjuvanticity after priming and investigated the impact of heterologous prime-boost approaches on the vaccine-specific immune responses. The modulation of primary T and B cell responses was characterized in mice primed with the mycobacterial antigen H56 and different adjuvants. Combinations of adjuvanted-H56 priming with homologous or heterologous boosting, were also analysed. Vaccine formulations containing the liposome system CAF01 or a squalene-based adjuvant elicited a primary CD4+ T cell response skewed to Th1/Th17 and Th1/Th2, respectively. Induction of short-lived plasma cells and early H56-specific IgG were observed mainly in mice immunized with CpG or the squalene-based adjuvant, while all tested adjuvants promoted the germinal centre reaction but with different magnitude. Mice primed with CAF01, but not antigen alone, and boosted with homologous or heterologous formulations, showed a strong secondary CD4+ T cell response.
Computational analysis performed with the FlowSOM software (R package) allowed clustering of antigen-specific T helper cells in different polyfunctional subsets producing combinations of TNF-α, IL-2 and IFN-γ, that were observed only in mice primed with the adjuvanted-formulation, regardless of the booster formulation used. These results show that the immunological activity of different adjuvants can be characterised profiling early biomarkers after immunization and highlight the crucial role of the adjuvant in generating primary immune responses that can be recalled by boosting. These data could give an important contribution to the rational development of heterologous prime-boost vaccine immunization protocols.

P.D3.02.09

Immunogenicity of Leishmania extracellular vesicles in combination with CpG ODN as a vaccine against cutaneous Leishmaniasis

B. Geçkin[1], *I. C. Ayanoğlu*[1], *I. C. Yılmaz*[1], *E. M. İpekoğlu*[1], *I. Gürsel*[2], *M. Gürsel*[1];
[1]*Department of Biological Sciences, Middle East Technical University, Ankara, Turkey,* [2]*Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey.*

Introduction: Cutaneous Leishmaniasis (CL), also known as Aleppo sore is a neglected parasitic disease that presents as self-healing skin lesions or cause debilitating large chronic or recurring lesions. During 1990-2010, >46.000 new CL cases caused by 3 different Leishmania species have been identified in Turkey. Considering the vast number of refugees immigrating to Turkey from Syria where CL is highly endemic, a substantial increase in CL cases is anticipated in the near future. The absence of an available licensed vaccine coupled with the cost, toxicity and drug resistance associated with the pentavalent antimonials used for treatment, necessitates the development of an effective preventive vaccine. Herein, we explored the immune protective vaccine potential of Leishmania antigen-rich small vesicles (exosomes) secreted from parasites in combination with CpG ODN based vaccine adjuvants.
Materials & Methods: Soluble leishmania antigen (SLA) or parasite exosomes were isolated from *L. major* species. For vaccination, Balb/c mice were immunized twice with: heat-killed parasites, SLA or exosomes as such or in combination with CpG ODN based vaccine adjuvants. Leishmania antigen specific IgG levels were quantified from sera by ELISA. Th1/Th2/Th17 responses elicited by vaccine formulations were measured antigen stimulated splenocytes using cytometric bead array (CBA).
Results: The results suggest that SLA and exosomes are promising anti-Leishmania protective antigens and D-type CpG ODN is the most promising adjuvant.
Conclusion: The preliminary work indicates that parasite exosomes are promising antigen carriers but methods to inactivate vesicle-associated proteases may increase their immunogenicity.

P.D3.02.10

Pigs as translational model: vaccination by tattoo with DNA in nanoparticles

A. Hoek[1], *A. van Nes*[1], *V. P. Rutten*[1], *G. G. Kenter*[2], *J. F. Engbersen*[3], *C. A. Jansen*[1], *A. D. Bins*[4];
[1]*Faculty of Veterinary Medicine, Utrecht, Netherlands,* [2]*Netherlands Cancer Institute, Amsterdam, Netherlands,* [3]*MIRA institute for Biomedical Technology & Technical Medicine, Enschede, Netherlands,* [4]*Academic Medical Center, Amsterdam, Netherlands.*

DNA vaccination in the skin is an established vaccination method in small laboratory animals. Historically, mice have been used as a preclinical model for dermal vaccine delivery, but extrapolation to humans may be difficult. Since the resemblance between pig and human skin, we investigated whether pigs may be used as preclinical model for dermal vaccination. Nanoparticle pDNA formulations were selected based on transfection efficiency of *ex vivo* pig skin. A selection of nanoparticle pDNA formulations in combination with the naked pDNA vaccine was subsequently applied *in vivo* by tattooing. Uptake of vaccine encoded antigen by cells in the draining lymph node (dLN) was determined by flowcytometry. Finally, HA37 formulated Human Papilloma Virus (HPV) 16 E6E7 DNA vaccine, currently used in a human trial, and unformulated DNA vaccine were assessed for their ability to stimulate T cells. Formulation of pDNA in polyplex HA37 resulted in the highest dermal transfection efficiency in pigs *in vivo*. Although cells in the dLN were able to take up the antigen, high background in some of the sham vaccinated pigs complicated this analysis. Tattoo with either HA37 DNA or naked DNA increased the number of IFNγ+ cells in the blood which was observed from the second vaccination onwards. In conclusion, DNA vaccination in the skin of pigs results in local production of antigen, its uptake by cells in the dLN and the induction of IFNγ+ cells. This suggest that DNA vaccination in the skin of pigs is an efficient way to induce vaccine-specific T cell responses.

P.D3.02.11

Corpuscular carrier enhances immune response after conjunctival immunization with chlamydial outer membrane proteins

A. Inic-Kanada[1], *M. Stojanovic*[2], *E. Stein*[1], *I. Lukic*[2], *E. Ghasemian*[1], *A. Filipovic*[2], *R. Miljkovic*[2], *T. Barisani-Asenbauer*[1];
[1]*Medical University of Vienna, Vienna, Austria,* [2]*Institute of Virology, Vaccines and Sera, Belgrade, Serbia.*

Trachoma, caused by the intracellular bacterium *Chlamydia trachomatis* (Ct), remains the world's leading preventable infectious cause of blindness. Recent attempts to develop effective vaccines rely on modified chlamydial antigen delivery platforms. We therefore characterized immune responses after conjunctival immunization with a N-terminal portion (amino acid 1-893) of the chlamydial polymorphic membrane protein C (PmpC) and a major outer membrane protein (MOMP) expressed in *Escherichia coli* bacterial ghosts (BGs) in a guinea pig model of ocular infection animals. Three immunizations were performed at two-week intervals, and the IgG immune responses were evaluated. Animals were further infected with *C. caviae* ($1x10^4$ IFU/animal) two weeks after the last immunization and ocular pathology and chlamydial clearance were investigated. Antigen-specific IgG levels in sera yielded significantly increased levels in the group immunized with MOMP BGs compared to animals immunized with PmpC BGs. Furthermore, a decrease in intensity of the transitional inflammatory reaction in conjunctiva of challenged guinea pigs immunized with MOMP-BGs compared with PmpC-BGs-immunized animals was observed. Analysis of changes in chlamydial load during the post-infection period revealed significantly lower absolute numbers of *C. caviae* in MOMP-BGs immunized guinea pigs on day 4 post-infection ($P < 0.05$) compared to the corresponding PmpC-BGs-immunized animals. Although neither immunization scheme provided full protection, MOMP-BGs merit further investigations as chlamydial protein carriers with intrinsic adjuvant properties in conjunctival vaccine against Chlamydia.

P.D3.02.12

Variance and dynamism of humoral responses in humans against a novel intranasal RSV subunit vaccine

M. S. Kalyan[1,2], *S. Ascough*[1,2], *I. Vlachantoni*[2], *B. Jan Haijema*[3], *S. Wallin*[3], *M. Dijkstra*[3], *M. S. Ahmed*[4], *Q. Zhang*[4], *K. Leenhouts*[3], *P. Openshaw*[2], *C. Chiu*[1];
[1]*Section of Infectious Diseases & Immunity, Imperial College London, London, United Kingdom,* [2]*National Heart and Lung Division, Imperial College London, London, United Kingdom,* [3]*Mucosis B.V. , represented by trustee Mr. Holtz LLM, Bout Advocaten, Groningen and Virtuvax B.V., Odijk, Netherlands,* [4]*Department of Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom.*

Respiratory syncytial virus (RSV) is a major cause of respiratory illness in both infants and the elderly but despite decades of research, no effective vaccine exists. We recently showed in human RSV infection challenges that mucosal IgA associated more closely with protection from infection than any other recognised correlate. Intranasal vaccination to induce both mucosal and systemic immunity could therefore offer preferential protection against RSV and other respiratory pathogens.
Here, we report humoral and cellular immunity data from a randomised controlled Phase 1 trial of an intranasal subunit vaccine comprised of the RSV F protein linked to a bacterium-like particle (BLP). The vaccine was well tolerated and significantly induced antibody-secreting cells, which were associated with consistent increments in anti-F protein serum IgG. Nasal IgA responses displayed heterogeneity in timing and magnitude, but very large increments seen in those with lower pre-existing titres. However, serum antibodies were preferentially non-neutralising and antibodies directed against the pre-fusion F protein conformation were not detectable. *In vitro* culture of tonsillar mononuclear cells from adults and children with the vaccine induced anti-F protein antibodies in a dose-dependent manner and was associated with marked CD4+ and CD8+ T cell proliferation with production of IFN-γ, IL-21 and IL-22.
Thus, this novel intranasal BLP platform can stimulate both mucosal and systemic responses, with serum antibody and plasmablast response comparable to live attenuated virus vaccines. The added advantages of subunit vaccine manufacturing and stability suggest that this vaccine can be a candidate for further optimisation.

P.D3.02.13

Innate immune responses to improve anti-carbohydrate vaccines against bacterial pneumonia

P. Kaplonek[1,2]*, L. E. Sander*[3]*, P. H. Seeberger*[1,2]*;*
[1]Max Planck Institute of Colloids and Interfaces, Potsdam, Germany, [2]Freie Universität Berlin, Institute of Chemistry and Biochemistry, Berlin, Germany, [3]Medizinische Klinik m.S. Infektiologie und Pneumologie, Charité Universitätsmedizin, Berlin, Germany.

High mortality rates of bacterial pneumonia and increase of antibiotic resistance is the major reason to develop novel vaccine strategies against *Streptococcus pneumoniae.* Ideal vaccines should be affordable, broadly available, easy to administer and induce long-lived protective immunity which requires activation of both innate and adaptive immune responses. Efficiency of vaccine depends on the quality and way of recognition of antigens as well as on proper adjuvants.
Preliminary results from our group showed protective effects of a SP3-tetra (the synthetic repeating unit tetrasaccharide of the *S. pneumoniae* serotype 3 (SP3) capsular polysaccharide) CRM_{197} conjugate vaccine *in vivo*, but long-term protection was limited. New adjuvants, such as prokaryotic mRNA and other vita-PAMPs (viability-associated pathogen-associated molecular patterns), have been shown to promote long-lasting humoral immunity in mice. Innate recognition of bacterial viability efficiently promotes human T_H1 and T_{FH} immunity. Thus, microbial signals (e.g. vita-PAMPs) that mimic live bacteria would represent ideal adjuvants for carbohydrate-conjugate vaccines directed against capsulated extracellular pathogens.
We design PRR (pattern recognition receptor)-ligand based adjuvant formulations to selectively induce innate signals that promote Th_1 and T_{FH} differentiation and humoral immunity. Encapsulated purified PAMPs (e.g. bacterial mRNA), or synthetic ligands (e.g. Imiquimod) in poly(lactic-co-glycolic acid particles (PLGA) are used to improve the immunoprotective effect of SP3-tetra in conjugation to both CRM_{197} and conserved pneumoccocal proteins (single amino acid substitution mutants, L469E, of pneumolysin). We use an *in vivo* mouse model (C57BL/6 mice) immunized with SP3-tetra conjugate microparticles.

P.D3.02.14

A reverse vaccinology approach in *Leishmania infantum* genome for the identification of surface-exposed/secreted antigens and selection of antigenic T cell epitopes for application in a multi-epitope peptide vaccine against leishmaniasis

M. Agallou[1]*, D. Zisis*[2]*, A. G. Hatzigeorgiou*[2,3]*,* ***E. Karagouni***[1]*;*
[1]Laboratory of Cellular Immunology, Department of Microbiology, Hellenic Pasteur Institute, Athens, Greece, [2]Bioinformatics-lab, Hellenic Pasteur Institute, Athens, Greece, [3]DIANA-Lab, Department of Electrical and Chemical Engineering, University of Thessaly, Volos, Greece.

Reverse vaccinology is a promising approach for direct screening of genome sequence assemblies for designing of new vaccine candidate proteins against different pathogens. The aim of the present study was to combine robust *in silico* approaches in the search for potential immunogenic T cell epitopes, based on the proteome of *Leishmania infantum*, for the development of an anti-*Leishmania* vaccine. Thus, the predicted proteome of *Leishmania infantum* was compared with other *Leishmania* species through BLAST protein alignment tool in order to select highly conserved protein sequences (>90%) resulting to a stringent set of 10 proteins. Then, subcellular and extracellular localization predictions were performed using Vacceed tool in order to select only the secreted and transmembrane proteins. Seven proteins were selected among which were 4 hypothetical proteins, 1 protein of unknown function and 1 protein namely vacuolar ATPase, subunit H. IEDB consensus tool was used to predict epitopes from the above proteins able to bind to MHCI and MHCII for the most prevalent allele supertypes of human and mice, whereas epitopes showing conservancy with human and murine proteome were excluded so as to avoid autoimmune responses. The resulting epitopes were then clustered to avoid redundancies and were used for molecular modelling and docking with MHCI and MHCII in order to select the most promising ones. *This research was funded by the grant from the Stavros Niarchos Foundation to the Hellenic Pasteur Institute and by EPAnEK Operational Programme (NSRF 2014-2020) co-financed by Greece and the European Union (T1EDK-03902).*

P.D3.02.15

Development of vaccine against fowl adenovirus 4

G. Ke[1]*, H. Chaung*[2]*, Y. Lien*[2]*, M. Wu*[3]*, T. Tsai*[1]*;*
[1]Graduate Institute of Animal Vaccine Technology, College of Veterinary Medicine/ Center of Animal Biologics, Pingtung, Taiwan, [2]Department of Veterinary Medicine, College of Veterinary Medicine/ Center of Animal Biologics, Pingtung, Taiwan, [3]Department of Food Science/ Research Center of Animal Biologics, Pingtung, Taiwan.

Fowl adenovirus-4 (FAdV4) belongs to avian adenovirus group I and is a double-stranded DNA virus without envelope. The diameter is about 70-90 nm. Clinical symptoms of FAdV4 infection is mainly Hydropericardium Syndrome (HPS) and Inclusion Body Hepatitis (IBH). HPS and IBH have a short incubation period and acute onset. The virus quickly spreads through the whole organism, resulting in a large number of deaths and huge economic losses. In this study, the FAdV4 virus was cultured with chicken liver cells and was successfully passaged in LMH cells. Titer of the virus gradually increased, reaching $10^{7.6}$ TCID50/mL at passage 10. The Hexon protein gene of FAdV4 was selected and expressed using the E. coli expression system, in which the protein expression reached 180 μg/mL. The inactivated 10^5 TCID50 FAdV4 virus along with 50 μg/mL recombination Hexon protein and adjuvant were prepared as a vaccine and used to immunize chickens. The neutralizing antibody titer reached over 2048x dilution 14 days after immunization. When immunized chickens were challenged, complete protection was observed and no virus was detected 14 days after challenge. The present results revealed that FAdV4 virus and Hexon protein retains high antigencity and can elicit high neutralizing antibody titers Therefore, it suggests that Hexon protein of FAdV4 could be a good candidate to be developed as a vaccine in the future.

P.D3.02.16

Virus like particles for the characterization of flavivirus cross-reactive antibody responses

E. Montoya-Diaz, *V. von Messling;*
Veterinary Medicine Division, Paul-Ehrlich-Institut, Langen, Germany.

The flavivirus family includes several important human pathogens. Due to the considerable structural and functional similarities, antibody responses induced by one virus frequently cross-react with other flaviviruses. Such cross-reactive antibodies are often beneficial but may also result in enhanced disease. A better understanding of the underlying mechanisms is thus essential for the development of safe vaccines in areas where several flaviviruses coexist. Since virus-like particles (VLPs) retain the morphology of the viral particle but are no longer infectious, they are a promising platform for flavivirus vaccine development. To assess the extent of flavivirus cross-reactive VLP-induced antibody responses and the associated risk for antibody-dependent enhancement of disease, we expressed the structural genes of Zika virus, Japanese Encephalitis virus, and Yellow Fever virus in mammalian cells and established a purification and concentration process, which reliably yields VLPs at concentrations around 1mg/ml. Immunization studies in mice to determine the kinetics of total and functional antibody responses against the homologous, as well as heterologous viruses with differing phylogenetic distance, are currently ongoing, and *in vitro* assays for the analysis of antibody-dependent enhancement activity are under development. We expect that these studies will provide new insights into the beneficial and adverse effects of cross-reactive flavivirus antibodies and contribute to the development of safe vaccines for these viruses.

P.D3.02.17

Intranasal immunization with outer membrane vesicle pertussis vaccine results in optimal protection

R. Raeven, ***D. Rockx-Brouwer****, L. van der Maas, T. Bindels, E. van Riet, B. Metz, G. Kersten;*
Intravacc, Bilthoven, Netherlands.

Immunization against *Bordetella pertussis* has led to a dramatic decrease of pertussis cases worldwide. Unfortunately, the world is currently facing a resurgence of whooping cough. This situation has led to the initiation of the development of improved pertussis vaccines. Our vaccine based on *B. pertussis* outer membrane vesicles (omvPV) is one of them. Subcutaneous immunization with omvPV leads to a broad antibody response against multiple antigens and a mixed Th1/Th2/Th17 response. While this systemic immunity is protective, we found that additional induction of mucosal immunity upon pulmonary administration of the vaccine improves protection against *B. pertussis* in mice. To explore alternative mucosal routes, we compared intranasal and subcutaneous omvPV immunization in mice to investigate the changes in protection and immunity. Intranasal immunization led to improved protection, and, in contrast to subcutaneous vaccination, induced strong mucosal responses of both IgA and Th17, while maintaining a similar degree of systemic IgG antibody levels, IgG-producing plasma cells, memory B-cells, and Th1/Th2/Th17 response. This study demonstrates that the development of mucosal pertussis immunity can be achieved by intranasal immunization and that this leads to enhanced protection against *Bordetella pertussis*.

P.D3.02.18

Identification of human Rhinovirus virus (HRV) CD8 T cell epitopes

M. Gomez Perosanz*, J. Sanchez-Trincado, M. Moreno Abraham, E. M. Lafuente, P. A. Reche Gallardo;*
Laboratory of Immunomedicine. Faculty of Medicine. Complutense University of Madrid, Madrid, Spain.

Adaptive immunity is based on the ability of T- and B-lymphocytes to recognize and remember antigens. More specifically, T- and B-lymphocytes recognize regions of the antigens known as epitopes. The knowledge of the epitopes that focus the attention of the immune system has a great practical relevance, as it allows to develop vaccines with a selective and controlled specificity. However, the development of this type of vaccine is not yet possible for certain pathogens whose epitopes are not described, as is the case of the human Rhinovirus (HRV). Here, we used a computational approach to select possible HRV epitopes from species A and C, the most frequent subtypes in the clinic of various pulmonary diseases. This approach is based on the identification of peptides within the conserved regions that show high HLA I-binding promiscuity.

Experimental identification of conserved CD8 T epitopes, was studied by IFNγ-ELISPOT assays using peripheral mononuclear cells (PBMC) from typed subjects exposed to synthetic peptides, consisting of predicted HRV-specific CD8 T epitopes. We have characterized 39 candidate epitopes derived from the computational analysis (25 of RVH A and 14 of HRV C) and 20 of them obtained a positive result in the analysis of release of IFNγ by ELISPOT. We verified peptide-binding to HLA-A0201 of ELISPOT positive responses using TAP-deficient T2 cell line binding-assays, which has led to the experimental identification of two Rhinovirus epitopes (VLEKGIPTL and GLEPLDLNTSAGFPYV) restricted by this allele that could potentially serve for the development of a vaccine.

P.D3.02.19

Optimization of HIV-1 DNA vaccine by comparing Minicircle and conventional vectors

***H. Huang**, W. Yikchun, C. Zhiwei, C. Samantha, C. Meisum;*
AIDS institute, Hong Kong, China.

DNA-based vaccine has become a promising vaccine vector because of its good safety profile and high immunogenicity. However, many current DNA vaccines only support short-term antigen expression and have low protective efficacy. Part of the reason is due to their large sizes and the presence of bacterial backbone sequences. Minicircle DNA vector is a next generation circular DNA vector that mostly consists of a small expression cassette, without the unnecessary bacterial backbone. Several studies have reported that minicircle vector enhances expression level and duration of inserted transgenes.
In this study, we examined the efficacy of minicircle vector in comparison to conventional pVAX plasmid as a vaccine against a HIV antigen in mouse models. The recombinant antigen focused in this study consisted of a soluble program death 1 (PD-1) molecule fused to a mosaic HIV-Gag-p41 antigen that was designed *in silico* based on the major circulating HIV sequences in China. The soluble PD-1 molecule allows effective antigen targeting to dendritic cells for antigen presentation.
We firstly confirmed the long-term antigen expression in mice treated with minicircle DNA vaccine intramuscularly with electroporation. Mice immunized with the minicircle vaccine induced a trend of higher $CD8^+$ in the spleen, lung and genital tract than those injected with an equal mass of the full-length pVAX vaccine during both acute and memory phases.
More importantly, the minicircle vaccine conferred a better protection in mice against intercostal challenge with malignant mesothelioma AB1 cells expressing HIV-Gag antigen. Collectively, our results demonstrate that minicircle vaccine is a favorable vaccine vector for inducing strong protective T cell immunity.

P.D3.02.20

Autoreactive potential of universal influenza vaccines

*M. R. Pillai[1], T. Chang[1], J. Crawford[1], R. Keating[1], C. Lewis[1], J. Labombarde[1], Q. Li[2], P. G. Thomas[1], **M. A. McGargill**[1];*
[1]St. Jude Children's Research Hospital, Memphis, United States, [2]UT Southwestern, Dallas, United States.

A universal influenza vaccine could save millions of lives in the event of a deadly pandemic. It is not clear why antibodies specific for conserved regions of influenza viruses are so rare. One possibility is that these antibodies have a higher potential to cross-react to self-proteins, and therefore B cells that generate these antibodies are deleted through tolerance mechanisms. In support of this, infections and vaccinations with the 2009 H1N1 pandemic strain induced more antibodies that were cross-reactive against multiple influenza strains than were induced by previous seasonal strains. However, they were also associated with a higher risk of autoimmune disorders, including narcolepsy and Guillain-Barré syndrome. Therefore, we examined whether cross-reactive influenza antibodies had a higher potential to be autoreactive than antibodies specific for one subtype of influenza. We previously demonstrated that H3N2-vaccinated mice treated with a low dose of rapamycin had more cross-reactive influenza antibodies and were better protected against subsequent lethal infections of multiple subtypes. Thus, we utilized rapamycin to increase the frequency of influenza cross-reactive antibodies, and tested whether these antibodies were more reactive to self-proteins than strain-specific antibodies. We found that mice with increased levels of cross-reactive influenza antibodies also had more IgM antibodies specific for self-antigens. Although the increase in autoreactive IgM antibodies was transient, it correlated with increased susceptibility to disease in mouse models of multiple sclerosis and Guillian-Barre Syndrome. Together, our results suggest that influenza cross-reactive antibodies have the potential to be autoreactive. These data have important implications for developing universal influenza vaccines designed to generate durable influenza cross-reactive antibodies.

P.D3.02.21

PHASE I, DOUBLE-BLIND, PLACEBO-CONTROLLED, RANDOMIZED SAFETY AND IMMUNOGENICITY TRIAL OF REACTIVATION OF PERTUSSIS TOXIN IMMUNITY WITH AN INVESTIGATIONAL EPICUTANEOUS PATCH IN HEALTHY ADULTS.

***O. Chatzis**[1], G. Blanchard-Rohner[1,2], L. Mondoulet[3], B. Pelletier[3], A. de Gea-Hominal[1], M. Roux[3], A. Huttner[4,1], P. L. Hervé[3], M. Rohr[2], A. Matthey[5], G. Gutknecht[5], B. Lemaître[6], C. Hayem[3], H. T. Pham[7], W. Wijagkanalan[7], P. H. Lambert[1], P. H. Benhamou[3], C. A. Siegrist[1,2];*
[1]Center for Vaccinology, University Hospitals of Geneva, Geneva, Switzerland, [2]Division of General Pediatrics, Department of Pediatrics, University Hospitals of Geneva, Geneva, Switzerland, [3]DBV Technologies, 177-181, avenue Pierre Brossolette, 92120, Montrouge, France, [4]Division of Infectious Diseases, University Hospitals of Geneva, Geneva, Switzerland, [5]Center for Clinical Research, University Hospitals of Geneva, Geneva, Switzerland, [6]Laboratory of Vaccinology, University Hospitals of Geneva, Geneva, Switzerland, [7]BioNet-Asia Co., Ltd., Bangkok, Thailand.

Background: Novel immunization strategies against pertussis are needed as immunity induced by acellular vaccines can be short-lived. Additionally, vaccination with needle-less and adjuvant-free patches could expand the target population benefiting from immunization. Methods: A Phase I, double-blind placebo-controlled randomized trial assessed safety and immunogenicity of an epicutaneous patch, Viaskin, administering recombinant pertussis toxin (rPT), in healthy adult (last PT-vaccination>10 years). Viaskin patches of PT 25µg, 50µg or placebo were administered at day 0 and 42, applied directly on the skin (n=25, 25, 10) or after controlled epidermal laser-based skin preparation (n=5, 25, 12). The primary outcome was safety of Viaskin PT. Antibody responses were assessed at day 14, 28, 42 and after administration of Boostrix®dTpa on day 70. Results: Baseline characteristics were similar among groups. Safety and tolerability profiles were favorable. Application directly on skin generated no detectable immunogenicity signal.In the group treated with Viaskin following skin preparation, D42 PT-IgG Geometric Mean Concentrations (GMCs) were significantly higher compared to placebo ($p<0.001$): Viaskin PT25 (33.24 IU/ml (95% CI, 9.59; 115.23), Viaskin PT50 (57.00 IU/ml (95% CI, 41.39; 78.51), placebo (4.03 IU/ml (95% CI, 2.56; 6.37). D42 seroconversion rates were significantly higher with Viaskin PT25 and PT50 vs. placebo (80% and 88% vs. 0%, $p=0.002$ and $p<0.001$, respectively). One-month after Boostrix®dTpa, PT-IgG antibody levels were similar in all groups.Conclusions: Viaskin PT applied after epidermal skin preparation resulted in favorable safety and immunogenicity: anti-PT booster responses were comparable to those elicited by Boostrix®dTpa.Clinical Trial Registration: NCT 03035370

P.D3.02.22

A replication-incompetent adenoviral vector-based HSV-2 vaccine induces strong humoral responses and protects mice against lethal HSV-2 challenge

***E. Rossetti**, M. Vujadinovic, E. van Huizen, M. Mulders, L. Tettero, J. Drijver, S. Damman, H. Schuitemaker, E. Saeland, R. Zahn;*
Janssen Vaccines and Prevention BV, Leiden, Netherlands.

Herpes Simplex Virus-2 (HSV-2) is the leading cause of genital herpes and a major global health problem. HSV-2 resides in nerve cells and can reactivate sub-clinically or cause genital blisters. Several prophylactic vaccines against this lifelong disease have been studied and failed in the clinic to show efficacy against the infection. Therefore, the development of an effective HSV-2 vaccine is urgently needed. Replication-incompetent adenoviral vectors are a very promising vaccine platform and they have shown to be safe and capable of inducing both humoral and cellular immune responses in animal models and in humans.
We have generated an adenoviral vector-based HSV-2 vaccine. Immunogenicity and efficacy of the vaccine were evaluated in an HSV-2 challenge model in mice. Virus neutralization and ELISA titers were measured in serum before challenge. Viral titers, clinical scoring and survival rate were monitored after intravaginal challenge with 200 LD50 HSV-2 G strain virus.
The HSV-2 vaccine candidate induced comparable high virus neutralizing antibody titers, both after prime and prime-boost immunization. Both vaccine regimens induced complete protection against lethal challenge with the wild type HSV-2 G strain virus. We are currently investigating HSV-2 latency in dorsal root ganglia isolated from the infected mice. Additionally, experiments are being conducted to further characterize the humoral response by measuring IgG subclasses and antibody dependent cellular cytotoxicity. Cellular responses will be measured by IFNγ-ELISpot and intracellular cytokine staining.
The results from this study strongly encourage us to investigate more thoroughly this promising vaccine platform against HSV-2.

P.D3.02.23

Education of Stem Cells by BCG: An innovative Approach in TB Vaccine Development

E. Kaufmann[1], *J. Sanz*[2], *J. L. Dunn*[1], *N. Khan*[1], *L. E. Mendonca*[1], *A. Pacis*[2], *F. Tzelepis*[1], *E. Pernet*[1], *A. Dumaine*[3], *J. Grenier*[3], *F. Mailhot-Léonard*[3], *E. Ahmed*[1], *J. Belle*[4], *R. Besla*[5], *B. Mazer*[1], *I. L. King*[1], *A. Nijnik*[4], *C. S. Robbins*[5], *L. B. Barreiro*[3], *M. Divangahi*[1];
[1]*McGill University Health Centre, Montreal, Canada,* [2]*Université de Montréal, Canada, Montreal, Canada,* [3]*Université de Montréal, Montreal, Canada,* [4]*McGill University, Montreal, Canada,* [5]*University of Toronto, Toronto, Canada.*

Introduction BCG is still the only available vaccine against TB, but prevents only the disseminated forms of the disease in early childhood. The efficacy of BCG against pulmonary TB in adults ranges from 0-80%. While control of TB requires T-cells to prevent disease progression, clinical trials of T-cell-targeting vaccines have failed to demonstrate protection against *Mycobacterium tuberculosis* (*Mtb*) infection. We therefore hypothesize that a protective mechanism afforded by BCG in adults is mainly dependent on monocytes/macrophages which are one of the first immune cells to encounter *Mtb* upon infection. However, considering the nature of monocyte/macrophage differentiation and their relatively short lifespan, we hypothesize that the access of BCG to the bone marrow (BM) will educate the hematopoietic stem cells (HSCs) to subsequently generate protective innate immunity.
Methods&Results Using mouse models, we demonstrated that following intravenous but not subcutaneous BCG vaccination, the bacteria access the BM. The presence of BCG in the BM significantly increased the numbers of lineage$^-$c-Kit$^+$Sca1$^+$ HSCs as well as multi-potent progenitors, and led to the generation of epigenetically modified monocytes/macrophages. By using parabiosis and chimeric mice as well as adoptive transfer approaches, we demonstrate that these educated monocytes/macrophages provide sustainable protection against *Mtb* infection *in vivo*.
Conclusions Our findings demonstrate that access of BCG into the BM is critical for generating a unique set of educated monocytes/macrophages that are protective against virulent *Mtb* infection. Reprogramming of HSCs thus may provide an innovative approach in vaccine development.
This work was supported by CIHR, DFG and FRQS.

P.D3.03 Novel approaches to vaccinology - Part 3

P.D3.03.01

***Bordetella pertussis* challenge fails to recall vaccine- and pre-exposure-induced circulating memory B cells**

M. Ballester[1,2,3], *B. Mastelic-Gavillet*[1,2,3], *P. Fontannaz*[1,4,3], *F. Auderset*[1,2,3], *P. Lambert*[1,2,3], *C. Siegrist*[1,4,3];
[1]*Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland,* [2]*World Health Organization Collaborating Center for Vaccine Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland,* [3]*Center for Vaccinology, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland,* [4]*World Health Organization Collaborating Center for Vaccine Immunology, Faculty of Medicine, Geneva, Switzerland.*

Despite decades of vaccine prevention, pertussis continues to occur and its prevalence is increasing. Clinical trials have demonstrated the efficacy of the current acellular vaccines (aP), however primo-infection still confers a better and more sustained protection. In humans, vaccine-induced antibodies rapidly wane after immunization, suggesting the failure to induce long-lived plasma cells. This contrast with murine data, in which antibody titres only slowly decline, in correlation with the persistence of plasma cells in the bone marrow. To circumvent the issue of antibody persistence in mice, we developed an adoptive transfer model in which memory immune cells induced by primo-infection or aP vaccination are transferred to naïve recipients prior to booster immunization or intranasal challenge with *Bordetella pertussis* (BP). This allows us to asses recall responses in the absence of circulating serum antibodies, mimicking the situation in humans.
Using this model, we found, that BP challenge recalls both vaccine-and pre-exposure-induced B cell memory much slower than a vaccine booster. As in humans, pre-exposure to BP provides a better protection than vaccine priming, efficiently initiating bacterial clearance in the first week post-challenge. This implies (1) that the delayed reactivation of memory B cells by BP contributes to the lack of protection against BP, and (2) that other key protective memory cells are induced by primo-infection but not aP vaccines.

P.D3.03.02

A prime with naked DNA improves the immune responses induced by a modified live vaccine against Porcine Reproductive and Respiratory Syndrome Virus

I. Bernelin-Cottet[1], *C. Urien*[1], *F. Blanc*[2], *V. Jakob*[3], *E. Bordet*[1], *C. Barc*[4], *V. Contreras*[5], *N. Bertho*[1], *C. Barnier-Quer*[3], *E. Studsrub*[6], *A. Brunsvik Fredriksen*[6], *H. Nauwynck*[7], *I. Schwartz-Cornil*[1];
[1]*VIM-INRA-Université Paris-Saclay, Jouy-en-Josas, France,* [2]*GABI-INRA-AgroParisTech-Université Paris-Saclay, Jouy-en-Josas, France,* [3]*Vaccine Formulation Laboratory, Department of Biochemistry, University of Lausanne, Epalinges, Switzerland,* [4]*INRA, UE1277, Plate-Forme d'Infectiologie Expérimentale, PFIE, Jouy-en-Josas, France,* [5]*CEA-Université Paris Sud-INSERM, U1184 « Immunology of viral infections and auto immune diseases », IDMIT department, IBFJ, Fontenay-aux-Roses et Kremlin-Bicêtre, France,* [6]*Vaccibody AS, Oslo, Norway,* [7]*VIM-INRA-Université Paris-SaclayLaboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium.*

The Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) induces reproductive disorders in sows and respiratory illnesses in growing piglets and is considered as the main pathogenic agent responsible for economic losses in the porcine industry worldwide. Modified live PRRSV vaccines (MLV) are by far the most efficient vaccine types but they are mainly protective against homologous strains and they may reverse to pathogenicity upon residual replication. We aimed at evaluating DNA vaccine strategies as stand-alone vaccines or used as priming to improve the MLV efficacy and safety. Our DNA PRRSV vaccines encode B and T antigens from a European subtype 1 strain which are relatively conserved across strains and which are expressed in a native form or in the form of vaccibodies targeted to the endocytic receptor XCR1 and CD11C expressed by dendritic cells. When delivered in skin with surface electroporation, the DNA vaccines induced antibody and T cell responses which were not promoted by antigen targeting to dendritic cells. Interestingly when delivered with surface electroporation or with needle-free jet fluid, a DNA prime enhanced the antibody response and broaden the T cell response induced by MLV. Furthermore, the DNA prime resulted in lower MLV replication in blood at day 20 after inoculation. The protective immunity against a heterologous strain is currently being evaluated.

P.D3.03.03

Development of a robust and standardized immunoserological assay for detection of anti-measles IgG antibodies in human serum

K. Böröcz*;*
Department of Immunology and Biotechnology, Clinical Centre, University of Pécs, Pécs, Hungary, Pécs, Hungary.

Introduction. Recent measles outbreaks face epidemiologists, diagnostic laboratories and health authorities with new challenges. High age-specific attack rates of the 1980-81 and 1988-89 epidemics suggest the existence of immunization gaps in the Hungarian population. Consequently, there is a need for continuous and large-scale monitoring of immunological protection against infection. For such monitoring to be feasible, a cost-effective, reliable and high-throughput assay is necessary. We developed a new ELISA protocol for assessment of anti-measles antibody levels in human serum.
Materials and Methods. A serum bank of anonymous patient sera was established (N >3000 samples). Sera were grouped based on measles immunization schedules and/or changes in vaccine components since the introduction of the first measles vaccine in Hungary in 1969. Our automated indirect ELISA was compared to indirect immunofluoresence and to anti-measles nucleocapsid (N) monoclonal antibody-based sandwich ELISA.
Results. Our results are in high agreement with the confirmatory methods (p-value< 0.0001). Based on measurement of 2057 sera, the highest ratio of low/questionable antibody level samples was detected in cluster '1978-1987' (~25.4%), followed by cluster '1969-1977' (~15.4%). Between age-groups of the new vaccination era, no statistical difference was found.
Conclusion. The identified potentially susceptible cohorts confirm our initial presumption of vaccine failure of the initial period of anti-measles immunization. Our new assay is cost-effective, allows high-throughput screening and has a better signal-to-noise ratio
compared to many commercial kits. This assay can serve as a first step in assessment of the effectiveness of all three components of the MMR vaccine.

P.D3.03.04

Development of vaccines against listeriosis

R. Calderón González*, E. Frande Cabanes, H. Terán Navarro, D. Salcines Cuevas, C. Álvarez Domínguez;*
Instituto de Investigación Marqués de Valdecilla (IDIVAL), Santander, Spain.

Introduction: Despite its increase in Europe during the last years, there is no vaccine approved against listeriosis. The goal of our laboratory has been the development of a prophylactic therapy that may be not only effective against the disease, but also easy and cheap to implement in healthcare systems.
Materials and Methods: While several *in silico* and *in vitro* experiments were made to search for peptides to be used in vaccination and to study its capacity to develop an immune response and its safety, the most useful tool in our research was the use of animal models. They allowed us to study the effectivity of our vaccines in models of different susceptibility to develop the disease, as well as listeriosis infection during pregnancy.
Results: The use of a cellular vector like dendritic cells allowed us to see that two peptides of *L. monocytogenes* LLO and GAPDH virulence factors have a great effectivity in the prevention of listeriosis, providing an effective Th1 immune response. As cellular therapy may be too expensive and difficult to apply on clinical practice, we use another vector: gold glyconanoparticles. These nanovaccines, used with adjuvants, proof to be as effective as dendritic vaccines, providing great protection levels in all the murine models we used.
Conclusions: Our laboratory has developed two different kinds of vaccines against listeriosis. While dendritic vaccines could be used in some specific cases like oncological patients, the use of nanovaccines may be a more convenient preventive therapy for the vaccination of global population.

P.D3.03.05

Increased adaptive immune responses and proper feedback regulation protect against clinical dengue

*E. Simon-Lorière[1], V. Duong[2], A. Tawfik[1], S. Ung[2], S. Ly[2], I. Casademont[1], M. Prot[1], N. Courtejoie[1], K. Bleakley[3], P. Buchy[2], A. Tarantola[2], P. Dussart[2], A. Sakuntabhai[1], **T. Cantaert**[2];*
[1]Institut Pasteur, Paris, France, [2]Institut Pasteur Cambodia/Institut Pasteur International Network, Phnom Penh, Cambodia, [3]INRIA Saclay, Palaiseau, France.

Clinical symptoms of dengue virus (DENV) infection, the most prevalent arthropod-borne viral disease, range from classical mild dengue fever to severe, life-threatening dengue shock syndrome. However, most DENV infections cause few or no symptoms. Asymptomatic DENV-infected patients provide a unique opportunity to decipher the host immune responses leading to virus elimination without negative impact on an individual's health. We used an integrated approach of transcriptional profiling and immunological analysis to compare a Cambodian population of strictly asymptomatic viremic individuals with clinical dengue patients. Whereas inflammatory pathways and innate immune response pathways were similar between asymptomatic individuals and clinical dengue patients, expression of proteins related to antigen presentation and subsequent T cell and B cell activation pathways was differentially regulated, independent of viral load and previous DENV infection history. Feedback mechanisms controlled the immune response in asymptomatic viremic individuals, as demonstrated by increased activation of T cell apoptosis-related pathways and FcgRIIB (Fcg receptor IIB) signaling associated with decreased anti-DENV-specific antibody concentrations. Together, our data illustrate that symptom-free DENV infection in children is associated with increased activation of the adaptive immune compartment and proper control mechanisms, leading to elimination of viral infection without excessive immune activation, with implications for novel vaccine development strategies.

P.D3.03.06

Age-dependent pre-vaccination immunity affects the immunogenicity of Varicella Zoster vaccination in middle-aged adults

*M. van der Heiden[1,2,3], **L. G. H. de Rond**[1], M. C. van Zelm[4,5], G. A. Berbers[1], A. M. Boots[2], A. Buisman[1];*
[1]National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands, [2]University of Groningen, Groningen, Netherlands, [3]The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden, [4]Erasmus MC, Rotterdam, Netherlands, [5]Central Clinical School, Monash University and Alfred Hospital, Melbourne, Australia.

Background The incidence of Herpes Zoster disease increases with age. Unfortunately, vaccine strategies to harness VZV-specific cell mediated immunity (CMI) demonstrate low effectiveness in the elderly, due to immune ageing. Timely vaccination of middle-aged adults might improve VZV-specific CMI at higher age. Therefore, we investigated immune responses after VZV vaccination (Zostavax) in Dutch middle-aged adults (N=53, 50-65 years of age). *Methods* Blood samples were taken pre-, 14 days, 28 days, and 1 year post-vaccination. VZV-specific IFNγ-producing cells were measured by Elispot, activated T-cells by flow cytometry, IgG and IgA antibody levels by fluorescent bead-based multiplex immunoassays, and whole blood cellular kinetics by TruCOUNT analysis. *Results* Robust short-term VZV-specific immune responses were observed post-vaccination. Remarkably, long-term enhancement of VZV-specific IFNγ producing cell numbers was induced only in participants with low pre-vaccination numbers of these cells, who were significantly older. These participants also showed enhancement of VZV-specific IgA 14 days post-vaccination. Finally, a high CD4/CD8 T-cell ratio was positively related to vaccine responsiveness. *Conclusion* These results suggest that adults in their early sixties, who showed a high CD4/CD8 T-cell ratio and low VZV-specific CMI, benefit from VZV vaccination, also shown by a VZV-specific IgA response at day 14 post-vaccination. This provides important knowledge for strategies to strengthen VZV-specific immunity before reaching old age.

P.D3.03.07

Vaccine-induced immune responses in Gabonese infants

***M. Esen**[1,2,3], J. Flügge[1,3], J. Honkpehedji[2], E. Askani[1,2], M. Massinga Loembe[1,2,4], S. Brückner[1], M. Duali[2], J. Strunk[1], B. Mordmüller[1,2,3], S. T. Agnandji[1,2], B. Lell[1,2], P. G. Kremsner[1,2,3], A. A. Adegnika[1,2,3];*
[1]Institut für Tropenmedizin, Tübingen, Germany, [2]Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon, [3]German Center for Infection Research (DZIF), Tübingen, Germany, [4]Université des Sciences de la Santé, Libreville, Gabon.

Helminth infections are common in Sub-Saharan Africa. It is generally recognized that infected individuals show a bias towards a Th2 immune response. Most vulnerable to helminth infection are children and pregnant women. The consequences of parasite infection during pregnancy for the mother and particularly for the fetus and the newborn can be severe and may include late effects on immune response during acute infection and vaccination. From February 2014 until September 2016 we conducted a study to investigate the influence of maternal parasite infection during pregnancy on the immune system of their infants in Lambaréné, Gabon and surroundings (ClinicalTrials.gov Identifier: NCT02714348). Here we present data on the immune responses to vaccines given within the expanded program on immunization (EPI) in 123 infants born to helminth infected and non-infected mothers. Helminth infection was diagnosed microscopically by the Kato-Katz method. Antibody titers to different vaccines (diphtheria, whooping cough, tetanus, poliomyelitis, hepatitis B and *Haemophilus influenza* A) were measured using commercial and validated ELISA kits. Our data show that infection with helminths is still common in pregnant women in Gabon but has only subtle effects on infants' immune responses to vaccines given as part of the EPI. Funding: This work was supported by the German Federal Ministry of Education and Research [01 KA 1009].

P.D3.03.08

SapM mutation to improve BCG vaccine efficacy: impact on vaccine clearance and quality of adaptive immune response

***N. Festjens**, K. Vandewalle, E. Houthuys, E. Plets, D. Vanderschaeghe, K. Borgers, A. Van Hecke, P. Tiels, N. Callewaert;*
VIB-UGent, Zwijnaarde, Belgium.

The *M. bovis* Bacille Calmette Guérin (BCG) vaccine shows variable efficacy in protection against adult tuberculosis (TB). Earlier, we have described a BCG mutant with a transposon insertion in the gene coding for the secreted acid phosphatase SapM, which led to enhanced long-term survival of vaccinated mice challenged with TB infection. We have now further characterized the genome and transcriptome of this *sapM*::Tn mutant versus parental BCG Pasteur. Moreover, we investigated the clearance of this improved vaccine strain from the immunization site, and the evoked immune response upon vaccination. Our findings strongly suggest that a more effective innate immune control over the vaccine bacteria leads to a more modest primary expansion of IFNγ Th1 and Tc1 cells. This correlates with an improved control of BCG *sapM*::Tn bacterial loads compared to WT BCG load following vaccination.

P.D3.03.09

DNA vaccination with APC-targeted hemagglutinin for prevention of influenza pandemics

***G. Grodeland**[1], T. Andersen[1], A. B. Fredriksen[2], B. Bogen[1];*
[1]University of Oslo and Oslo University Hospital, Oslo, Norway, [2]Vaccibody AS, Oslo, Norway.

Pandemic influenza represents a threat to society, and novel vaccine strategies are needed to counter an unexpected emergence. DNA vaccines can be rapidly manufactured, but it has been difficult to translate promising results from DNA vaccination in mice to larger animals and humans. Immunogenicity of DNA vaccination can be greatly increased by targeting of antigen to antigen presenting cells (APC). Further, by careful selection of receptor targets on APC, the induced immune responses can be polarized to different types of immunity. Thus, targeting of influenza hemagglutinin (HA) towards MHC class II (MHCII) molecules is particularly efficient for induction of rapid, enhanced and long-lasting antigen-specific antibody titers in both mice and larger animals. Correspondingly, targeting of HA to chemokine receptors particularly raise cellular immunity. In the context of influenza prevention, antibodies represent an important correlate of protection. Thus, we have developed novel HLA class II-targeted DNA vaccines that could be rapidly produced to counter a pandemic emergence. Importantly, the HLAII-specific targeting unit binds most of the polymorphic HLA class II molecules pan-specifically in humans. A single HLAII-targeted DNA vaccination induced neutralizing antibodies in larger animals such as ferrets, pigs and rhesus macaques. As an indication that the vaccines could also be useful for human application, HLA class II-targeted vaccine proteins were found to increase human $CD4^+$ T cell responses by a factor of $x10^3$ *in vitro*.

P.D3.03.10

Characterization of early responses in blood skin and lymph node compartment induced by intradermal injection of Modified Vaccinia Ankara

*P. Rosenbaum[1], N. Tchitchek[1], **C. Joly**[1], L. Stimmer[2,3], H. Hocini[4,5], N. Dereuddre-Bosquet[1], A. Beignon[1,4], C. Chapon[1,4], Y. Lévy[4,5], R. Le Grand[1,4], F. Martinon[1,4];*
[1]Immunology of Viral Infections and Autoimmune Diseases, IDMIT Department, CEA – Université Paris Sud 11 – INSERM U1184, Fontenay-aux-Roses, France, Fontenay-aux-Roses, France, [2]CEA – INSERM, MIRCen, UMS27, Fontenay-aux-Roses, France, Fontenay-aux-Roses, France, [3]INSERM U1169, Kremlin-Bicêtre, France, Kremlin-Bicêtre, France, [4]Vaccine Research Institute, Henri Mondor Hospital, Créteil, France, Créteil, France, [5]INSERM U955, Henri Mondor Hospital, University of Paris East, Créteil, France, Créteil, France.

Vaccine design approaches would be greatly facilitated by a better understanding of the early immune changes, and those that occur at the site of injection, inducing a durable and oriented protective response. We characterized in details very early infection and host response events after the intradermal administration of the modified vaccinia virus Ankara as a live attenuated vaccine model in non-human primates. We performed *in vivo* imaging, histology, flow cytometry, multiplex cytokine, and transcriptomic and analyzed data using tools derived from systems biology, such as co-expression networks.
We showed a strong early local and systemic inflammatory response that peaked at 24 h, which was then progressively replaced by an adaptive response during the installation of the host response to the vaccine. Granulocytes, macrophages, and monocytoid cells were massively recruited during the local innate response in association with local productions of GM-CSF, IL-1β, MIP1α, MIP1β, and TNFα. We also observed a rapid and transient granulocyte recruitment and the release of IL-6 and IL-1RA, followed by a persistent phase involving inflammatory monocytes. This systemic inflammation was confirmed by molecular signatures, such as up-regulations of IL-6 and TNF pathways and acute phase response signaling. Such comprehensive approaches improve our understanding of the spatio-temporal orchestration of vaccine-elicited immune response, in a live attenuated vaccine model, and thus contribute to rational vaccine development.

P.D3.03.12

Tannic acid-modified silver nanoparticles as novel adjuvants in herpes virus infection

*P. Orlowski[1], E. Tomaszewska[2], K. Ranoszek-Soliwoda[2], G. Celichowski[2], J. Grobelny[2], **M. Krzyzowska**[1];*
[1]Military Institute of Hygiene and Epidemiology, Warsaw, Poland, [2]Faculty of Chemistry, University of Lodz, Lodz, Poland.

Silver nanoparticles (AgNPs) are promising new antimicrobial agents against a wide range of skin and mucosal pathogens. We have previously shown that tannic acid modified silver nanoparticles (TA-AgNPs) consist a promising microbicide against genital herpes. The aim of this study was to study the ability of TA-AgNPs to induce DCs activation in the presence of HSV-2 antigens when used at non-toxic doses. Additionally, we tested ability of TA-AgNPs to induce efficient anti-viral immunity in vivo using mouse genital herpes model. Preparations of HSV-2 treated with nanoparticles (TA-AgNPs-HSV-2) were used to investigate HSV-2 antigen uptake, activation markers and cytokine production by JAWS II dendritic cell line. We also accessed proliferation and activation of HSV-2 specific T cells by DCs treated with TA-AgNP-HSV-2. Our results showed that TA-AgNPs were potent stimulators of DCs maturation and helped to internalise viral antigens. TA-AgNPs-HSV-2 also helped to overcome inhibition of DCs maturation by live or inactivated virus through up-regulation of activation markers and cytokine production. HSV-2 treated with TA-AgNPs stimulated activation of memory CD8+ T cells, and induction of IFN-γ producing CD4+ and CD8+ T cells. HSV-2 infected mice treated intravaginally with TA-AgNPs showed better recovery, lower viral titers in spinal cords, higher levels of seroneutralising antibodies upon viral challenge and development of effective B and T cell memory compartment. We conclude that TA-AgNPs consist a novel class of nano-adjuvants, which can help to overcome virus-induced suppression of DCs activation. This work was supported by DEC- 2012/05/N/NZ6/01757 grant.

P.D3.03.13

Circulating T follicular helper cells and immune response induced by influenza vaccine in children with acute lymphoblastic leukemia during maintenance therapy

***N. Le Corre**[1,2], C. P. Martinez-Valdebenito[1,2], F. Barriga[3], M. Contreras[3], M. Vidal[3], R. Moreno[4], X. Claverie[4], P. Contreras[4], L. Huneman[4], R. Alarcón[2], T. García[1], R. Rathnasinghe[1], R. Medina[1], M. Ferrés[1,2];*
[1]Dpto Enfermedades Infecciosas e Inmunología Pediátrica, Pontificia Universidad Católica de Chile, Santiago, Chile, [2]Laboratorio de Infectología y Virología Molecular, Red Salud UC-Christus, Santiago, Chile, [3]Unidad Oncología División Pediatría, Pontificia Universidad Católica de Chile, Santiago, Chile, [4]Unidad Hemato-Oncología Pediátrica Complejo Asistencial Dr. Sótero del Río, Santiago, Chile.

Vaccine immune response is impaired in immunocompromised patients. Follicular helper-T-lymphocytes (cTfh) are essential for high-affinity and long-lasting humoral response. We evaluate the role of cTfh in immune response induced by influenza vaccine in children with acute linfloblastic leukemia (ALL). Children with ALL in maintenance therapy and a control group of healthy children were included. Blood samples were taken on the day of vaccination (D0), and on day 28 (D28). The humoral response and frequency of cTfh were evaluated. Twenty-four children with ALL and 8 healthy children were included: 66,7 and 38% were women, median age of 5 years old in both. A 33,3%(8/24) of patients and 63%(5/8) of controls were seroprotected at D28. Seroprotected children were significantly older (10 years) than non-protected children at D28 (3.6 years),(p=0,004). During follow-up, three children had influenza infection. An increase of percentage of cTfh cells from D0 to D28 was observed in both groups, but only significantly in ALL (mean-ALL, D0-D28:18-23% (p=0.003) and mean-controls, D0-D28:22-26%).
Comparing seroprotected versus non-seroprotected children no differences were found in cTfh cell at D0 or D28. The increase of percentage of cTfh cells from D0 to D28 observed in both groups, was significant only non-seroprotected subject (mean-seroprotected, D0-D28:21-24%and mean-non-seroprotected, D0-D28:18-24% p=0.004).Children with ALL achieved a lower seroprotection than healthy children. After vaccination, all children had a increase of cTfh cells. We did not found association between percentage of cTfh cells and seroprotection at D28. It should be evaluated if the lack of humoral response is associated to cTfh disfunction. Fondecyt-11150970

P.D3.03.14

An Immunogenic Epitope Chimeric Protein of HAdV for Antibodies Detection as well as Immunity Analysis

***Y. Li**, Y. Qi, X. Wang, J. Li, J. Rao, W. Shen, W. Zeng, S. Liu, X. Li, Y. Lin;*
Huadong Research Institute for Medicine and Biotechniques, Nanjing, China.

<META NAME="author" CONTENT="Microsoft Office 用户">
To construct and express a chimeric protein of immunogenic epitopes from five types of human adenoviruses(HAdV), type 3, 7, 11, 14 and 55, and identify its immunoactivity. The amino acid sequences of hexons from the five types of HAdV were analyzed respectively by using software, and the immunogenic epitopes with strong antigenicity were screened. The selected immunogenic epitopes were linked together with Gly-Gly-Ser for constructing a chimeric protein of immunogenic epitopes. The DNA of the chimeric protein was synthesized chemically, and cloned into plasmid pET-28a(+) for expressing the chimeric protein. The chimeric protein was purified by Ni-NTA resin and used as an antigen to immune Balb/c mice, the antisera were prepared. The antigenicity and immunogenicity of the chimeric protein were detected by ELISA. The chimeric protein was expressed and purified successfully. The titers of antiserum from the mice immunized four times with the chimeric protein reached to 1:320,000, and the ELISA results confirmed that the chimeric protein has strong antigenicity and immunogenicity. The expressed chimeric protein of immunogenic epitopes from the five types of HAdV laid foundation for developing vaccine and diagnostic reagents.
Keywords: human adenovirus; chimeric protein; antigenicity; immunogenicity
Acknowledgements
(We acknowledge grant support from China Key Project of New Medicine Development (2015ZX09J15105-001-003); China Jiangsu Province Social Development Foundation (No. BE2016622).)
★: Corresponding author, Email: liyxi2007@126.com

P.D3.03.15

Repeated mycobacteria vaccination in mice induces myeloid-derived suppressor cell killing of splenic dendritic cells via iNOS-dependent NO production

*E. Ribechini[1], I. Eckert[1], A. Beilhack[1], N. Du Plessis[2], G. Walzl[2], U. Schleicher[3], U. Ritter[4], **M. B. Lutz**[1];*
[1]University of Würzburg, Würzburg, Germany, [2]Stellenbosch University, Cape Town, South Africa, [3]University of Erlangen, Erlangen, Germany, [4]University of Regensburg, Regensburg, Germany.

Myeloid-derived suppressor cells (MDSCs) accumulate in patients with tuberculosis (TB) and a vaccine based on *Mycobacterium tuberculosis* (Mtb) is lacking. From this, we hypothesized that Mtb-based vaccines may induce MDSCs impairing vaccination success. Our data indicate that *in vitro*, bone marrow-derived resting MDSC (R-MDSC) stimulation with heat-killed Mtb resulted in the production of NO, directly suppressing T cells and inducing bone marrow-derived dendritic cell (BM-DC) apoptosis. The killing was NO dependent since blocking of iNOS reverted the effect. *In vivo*, repeated immunization of mice with Complete Freund´s Adjuvant (CFA) containing Mtb but not Incomplete Freund's Adjuvant (IFA) lacking the Mtb component induced activated MDSCs (A-MDSCs) in the spleen. Myeloid cells isolated from spleens of CFA/CFA injected mice but not single CFA or CFA/IFA injected mice suppressed $CD4^+$ and $CD8^+$ T cell proliferation in a nitric oxide (NO) dependent manner. The accumulation of $Gr\text{-}1^+$ $CD11b^+$ $iNOS^+$ myeloid cells was restricted to the splenic red pulp and bridging channels. Short term microbial challenge of mice *in vivo* induced infiltration of $iNOS^+$ A-MDSCs after 6h into the white pulp resulting in conventional DCs (cDCs) and plasmacytoid DCs (pDCs) apoptosis in the T cell areas of the white pulp after 24h. DC apoptosis was not observed after microbial challenge alone and was reduced in $NOS2^{-/-}$ mice. In contrast, apoptosis of T cells was not observed and macrophage killing occurred but was independent of NO. Together, our data indicate that Mtb vaccines induced and activated MDSCs in spleens of mice leading to NO-dependent DC killing.

P.D3.03.16

Immune profile driven by a novel tuberculosis nanovaccine correlates with protection against *Mycobacterium tuberculosis* infection

***A. Martínez-Pérez**[1], A. Igea[1], O. Estévez-Martínez[1], C. M. Ferreira[2], A. G. Castro[2], E. Torrado[2], Á. González-Fernández[1];*
[1]Immunology. Biomedical Research Centre (CINBIO) (Singular Centre of Research), Galicia Sur Health Research Institute (IISGS), University of Vigo, Vigo, Spain, [2]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, and PT Government Associate Laboratory ICVS/3B's, Braga, Portugal.

Introduction: The mechanisms underlying protection against *Mycobacterium tuberculosis* (Mtb) infection remain unclear. Prime vaccination with BCG following by boosting actions with novel vaccines has emerged as a promising strategy. In this regard, a new vaccine composed by a nanoparticle and a fusion protein containing three Mtb antigens and administered intranasaly, has been shown to enhance protection against Mtb when compared to BCG alone. In this study, we aimed at defining the immune cellular and molecular profile generated by this vaccine with the ultimate goal of identifying correlates of protections against Mtb infection.
Materials and methods: Mice were vaccinated with BCG following by two boosts two weeks apart with two different nano-vaccines. Two weeks after the last boost, mice were sacrificed and lung and spleen cells analyzed by flow cytometry and RNA-Sequencing.
Results: We found different protection levels induced by the tested nanovaccines. Interestingly, the level of protection correlated with the polyfunctionality of the Ag-specific CD4 T cell response and composition of the lung immune infiltrates. Crucially, gene expression analysis revealed a unique profile of differentially expressed genes in protected mice.
Conclusions: The results obtained in this study offer new insights that may be useful in the design of novel and more efficient vaccines to tuberculosis.

P.D3.03.17

Targeting of influenza viral epitopes to antigen presenting cells by genetically engineered chimeric molecules in humanized NSG transfer model

N. Mihaylova[1], *I. Ivanova*[1], *I. Manoylov*[1], *D. Makatsori*[2], *S. Lolov*[3], *M. Nikolova*[4], *A. Mamalaki*[2], *J. Prechl*[5], *A. Tchorbanov*[1,6];
[1]*Laboratory of Experimental Immunology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria,* [2]*Hellenic Pasteur Institute, Ampelokipi, Athens, Greece,* [3]*Institute of Biology and Immunology of Reproduction, Bulgarian Academy of Sciences, Sofia, Bulgaria,* [4]*National Reference Laboratory of Immunology, NCIPD, Sofia, Bulgaria,* [5]*Immunology Research Group, Hungarian Academy of Sciences, Budapest, Hungary,* [6]*National Institute of Immunology, Sofia, Bulgaria.*

Introduction: Anti-viral DNA vaccines are a novel strategy in the vaccine-development field, which consists in the administration of expression vectors coding viral antigen sequences into the host's cells. It has been shown that FcγRI on human monocytes enhances antigen presentation *in vivo*. Targeting of conserved viral epitopes by antibody fragments specific to activating cell surface co-receptor molecules on antigen-presenting cells could be an alternative approach for inducing protective immunity. Materials and Methods: Genetic engineering, signal transduction, cell transfection, flow cytometry, generation of humanized NOD/SCID model, ELISpot, ELISA and cytotoxicity assays; Results: Various DNA constructs, encoding a scFv fragment from mouse anti-human FcγRI monoclonal antibody, coupled to a sequence, encoding a T- and B-cell epitope-containing influenza A virus hemagglutinin intersubunit peptide were inserted into the eukaryotic expression vector pTriEx-3 Neo. The constructed chimeric DNA molecules were expressed by transfected CHO cells and the ability of the engineered proteins to interact with FcγRI-expressing cells was confirmed by flow cytometry. The fusion protein induced a strong signal transduction on human monocytes via FcγRI. The expression vector pTriEx-3 Neo containing the described construct was used as a naked DNA vaccine and introduced directly to experimental humanized NOD/SCID mice with or without boosting with the expressed fusion protein. Immunization with the generated DNA chimeric molecules, and prime-boost with the expressed recombinant proteins induced significant serum levels of anti-influenza IgG antibodies and strong CTL activity against influenza virus-infected cells in humanized animals. Conclusions: Genetically engineered molecules elicit an efficient anti-influenza immune response in the humanized mice.

P.D3.03.18

T-CELL AND B-CELL MEMORY IMMUNITY AFTER A SINGLE DOSE BIVALENT HPV VACCINATION COMPARED TO TWO- OR THREE DOSE VACCINATED GIRLS TO 6 YEARS POST-VACCINATION

H. Pasmans[1], *T. Schurink- van ,t Klooster*[1], *M. Welters*[2], *S. van der Burg*[2], *F. van der Klis*[1], *A. Buisman*[1];
[1]*RIVM, Bilthoven, Netherlands,* [2]*LUMC, Leiden, Netherlands.*

Vaccines consisting of virus-like particles (VLPs) against Human Papillomavirus (HPV) are currently administered in subsequently two- or three injections. However, it has been shown that just one-dose of the bivalent HPV16/18 vaccine results in the seroconversion of all women in a 7 years follow-up vaccination study. The seroconversion is associated with a low prevalence of HPV-16/-18 infections suggesting that the induced protection after one-dose of vaccine may be long lived. Since T- and B-cell responses are important in the immunity against HPV, we evaluated these responses in subjects vaccinated with different schedules. Blood was cross-sectional collected and PBMCs were isolated from girls vaccinated at 12 years of age according to a one-, two- or three-dose schedule. T-cells producing IFN-γ were determined by ELISPOT after stimulation of PBMCs by VLPs for HPV-16, HPV-18, HPV-31 and HPV-45. HPV-type-specific memory B-cell responses were determined by specific ELISPOT assays after polyclonal stimulation of isolated (CD19+) B-cells. Even after 6 years following vaccination HPV-type-specific interferon-gamma producing T cells were detectable .
However, these numbers are lower compared to two- and three-dose vaccinated individuals. Memory B-cell responses are detected at least 2 years post vaccination against type 16, 18, 31 and 45. The one-dose schedule is highly immunogenic and induces long-term HPV-specific T- and B-cell memory, although further studies will evaluate T-cell responses in more detail. The results suggests that a booster immunization might not be necessary to induce long-lived HPV-specific immunity.

P.D3.03.19

Identification of candidate *Coxiella burnetii* T-cell epitopes for a novel human Q-fever vaccine

A. Scholzen[1], *L. Moise*[2], *G. Richard*[2], *P. M. Reeves*[3], *S. Raju Paul*[3], *T. A. Brauns*[3], *L. A. Baeten*[4], *R. A. Bowen*[4], *R. Bucala*[5], *C. M. Boyle*[2], *W. D. Martin*[2], *A. E. Sluder*[3], *A. Garritsen*[1], *A. S. De Groot*[2], *M. C. Poznansky*[3];
[1]*Innatoss Laboratories, Oss, Netherlands,* [2]*EpiVax, Providence, United States,* [3]*Massachusetts General Hospital, Boston, United States,* [4]*Colorado State University, Fort Collins, United States,* [5]*Yale University School of Medicine, New Haven, United States.*

Coxiella burnetii (Cb), the causative agent of Q-fever, is a Gram-negative intracellular bacterium transmitted via aerosol. Regulatory approval of the Australian whole-cell vaccine Q-Vax in the US and Europe is hindered by reactogenicity in previously exposed individuals. The aim of this study was to identify and rationally select Cb epitopes for a safe, effective and less reactogenic T-cell targeted human Q-fever vaccine. Immunoinformatic methods were used to predict 65 HLA class I and 50 class II Cb epitopes. HLA binding assays confirmed 89% of class I and 75% of class II predictions, and 11 class II epitopes elicited IFNγ responses following heterologous DNA/peptide prime-boost immunizations of tgHLA-DR3 mice. Individuals naturally exposed to Cb during the 2007-2010 Dutch Q-fever outbreak were divided into three groups: controls without immunological evidence for previous infection and individuals with responses to heat-killed Cb in a whole blood IFNγ release assay (IGRA) which remained asymptomatic or experienced clinical Q-fever. Recall responses to Cb epitopes were assessed by cultured IFNγ ELISpot. While class I epitope responses were sparse, we identified 21 class II epitopes that re-called T-cell IFNγ responses in 10-28% of IGRA+ subjects. Past asymptomatic and symptomatic IGRA+ individuals showed a comparable response pattern and cumulative peptide response which correlated with IGRA responses. These data demonstrate that a substantial proportion of immunoinformatically-identified class II epitopes show long-lived immunoreactivity in naturally infected individuals, making them desirable candidates for a novel epitope-based Q-fever vaccine. This work was supported by contract HDTRA1-15-C-0020 from the US Defense Threat Reduction Agency.

P.D3.03.20

Improving the vaccine efficacy of recombinant BCG utilizing the major membrane protein-II (MMPII) antigen against tuberculosis

Y. Tsukamoto[1], *Y. Maeda*[1], *T. Tamura*[1], *T. Mukai*[1], *S. Mitarai*[2], *S. Yamamoto*[3], *M. Makino*[1];
[1]*Department of Mycobacteriology, Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan,* [2]*Department of Mycobacterium Reference and Research, The Research Institute of Tuberculosis, Tokyo, Japan,* [3]*Japan BCG Laboratory, Tokyo, Japan.*

Mycobacterium bovis BCG (BCG) has been used as a vaccine against tuberculosis. However, its efficacy against adult pulmonary tuberculosis is limited. To improve the efficacy of BCG vaccination against tuberculosis, we utilized Major Membrane Protein-II (MMPII) antigen (Ag) from *M. tuberculosis* (MTB). MMP-II Ag is highly immunogenic in terms of activating T cells in vitro. We developed a new recombinant BCG vaccine against MTB with MMP-II Ag from MTB, termed as BCG-DHTM. BCG-DHTM has two characteristics; (1) expresses HSP70-MMPII fusion Ag and (2) induces phagosome-lysosome fusion in cells infected with BCG-DHTM due to depletion of *UreC* gene in host BCG. BCG-DHTM secreted the fusion Ag and efficiently stimulated immune cells. To improve the vaccine efficacy of BCG-DHTM, we added proteolysis-inducing signal (PEST sequence) on both ends of HSP70-MMPII fusion gene and introduced into *ureC*-gene depleted BCG, and termed this BCG as BCG-PEST. BCG-PEST secreted the PEST-HSP70-MMPII-PEST fusion Ag and more efficiently induced cytokine production from human APCs than control BCG. DCs infected by BCG-PEST effectively activated naive T cells and promoted IFN-γ production. Furthermore, a single inoculation of BCG-PEST more effectively reduced the multiplication of MTB in murine lungs than control BCG. These results suggest that vaccination with BCG-PEST may efficiently control the growth of MTB in human.

P.D3.03.21

Cellular immune responses to influenza vaccination in a Dutch cohort of healthy individuals

S. Rosendahl Huber[1], *A. Turksma*[2], *M. Hendriks*[1], *R. H. Jacobi*[1], *R. A. van Boxtel*[1], *N. Y. Rots*[1], *A. ten Brinke*[2], *W. Luytjes*[1], ***J. van Beek***[1];
[1]*National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands,* [2]*Sanquin Research, Amsterdam, Netherlands.*

Introduction: Influenza virus-specific T cells are able to limit influenza virus infection and reduce clinical symptoms. However, the induction of T cells by current influenza vaccines remains under debate. **Materials and Methods:** We performed a vaccine trial in adults and collected PBMCs before, after vaccination, and after 2nd and 3rd vaccinations in two consecutive seasons (2009 - 2011). We analyzed the T cell responses by IFN-γ ELISPOT. PBMCs were stimulated for 18 hours with live influenza A virus or the vaccine components HA or NA. The data was analyzed in a mixed effects negative binomial regression statistical model to correct for pre-existing immunity, sex and age. In depth analyses was performed by FluoroSpot and flow cytometry for CD40L in combination with different cytokines. **Results:** We showed that a single dose of unadjuvanted or adjuvanted vaccine resulted in a significant induction of the response. Interestingly, the revaccination with an unadjuvanted vaccine 1 year after a adjuvanted vaccination induced a significant additional increase in cellular levels compared to the post-vaccinations level, whereas, a 2nd vaccination, three weeks after the first dose, did not result in a further increase.
In depth analysis by flow cytometry and FluoroSpot using the vaccine antigens HA and NA confirmed vaccine specificity, activation status and Th1 cytokine profile of the T cell response. **Conclusion:** We show the induction of T cells by both adjuvanted and unadjuvanted subunit influenza vaccination. Research was funded by the Dutch government

P.D3.03.22

The cAMP CHO reporter cell line to replace the *in vivo* safety test for acellular pertussis vaccines

*M. E. Hoonakker, L. M. Verhagen, **J. van der Cruijsen**, L. de Brouwer, L. van der Maas, A. Sloots, C. F. Hendriksen;*
Intravacc, Bilthoven, Netherlands.

Pertussis toxin (PTx) is one of the major virulence proteins of *Bordetella pertussis*. Since pertussis toxoid (PTd), the detoxified form of PTx, contributes to protection, this toxoid is the key component of all acellular pertussis vaccines. To examine possible toxin content in vaccine batches, each batch is subjected to the *in vivo* Histamine Sensitization test (HIST). In the last decades the intrinsic limitations of this test - including a lack of mechanistic understanding and animal welfare concerns - have pushed the search for alternative methods. A promising alternative method is based on PTx-induced clustered growth of CHO cells, though the subjective read-out and the limited capacity to discriminate between levels of clustering hamper quantitative detection of PTx levels. On a cellular level, PTx primarily interferes with intracellular pathways that involve cAMP. Based on this phenomenon, we generated a CHO reporter cell line that stably expresses a reporter construct responsive to changes in intracellular cAMP levels. This cell line enables the detection of PTx in a concentration-dependent manner up to a concentration well below the levels detected with the *in vivo* HIST. More importantly, the cell line detects PTx in the context of an aluminium phosphate adjuvated aP multivalent vaccine, with a sensitivity equal to the HIST. These results demonstrate that the CHO reporter cell line enables simple, quantitative and concentration-dependent detection of PTx. The cell line therefore offer a promising *in vitro* method to replace the suboptimal *in vivo* HIST and *in vitro* CHO clustering tests.

P.D3.03.23

Chitosan mediated co-delivery of SN38 and Snail-specific siRNA as a useful anticancer approach against prostate cancer

***V. Younesi**[1], A. Afkham[2], L. Aghebati-Maleki[2], H. Siahmansouri[3], M. Ahmadi[4], M. Yousefi[5];*
[1]Tabriz University Medical Of Sciences, Tabriz, Iran, Islamic Republic of, [2]Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, Tabriz, Iran, Islamic Republic of, [3]Department of Immunology, Tabriz University Medical Of Sciences, Tabriz, Iran, Islamic Republic of, [4]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, Tabriz, Iran, Islamic Republic of, [5]Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran, Tabriz, Iran, Islamic Republic of.

Background: Prostate cancer is known as the most common malignancy in men. Chitosan has generated great interest as a useful biopolymer for the encapsulation of small interfering RNA (siRNA). Due to cationic nature, chitosan is able to efficiently encapsulate siRNA molecules and form nanoparticles. Furthermore, the biocompatible and biodegradable attributes of chitosan have paved the way for its potential application in the in vivo delivery of therapeutic siRNAs. In this study, we aimed to design chitosan/CMD nanoparticles for the efficient encapsulation of the anti-cancer drugs SN38 and Snail-specific siRNA.
Methods: Physicochemical characteristics of the synthesized chitosan nanoparticles were analyzed using Scanning Electron Microscopy. Down regulation of targeted genes were confirmed using real time PCR assays. Growth inhibitory properties of the Dual delivery of SN38-Snail siRNA CMD-chitosan nanoparticles were investigated by MTT assay in metastatic prostate cancer cells. Results: Our findings revealed that in ChNP-CMD-SN38-siRNA treated cells the mRNA level of snail decreased from 1.00 to 0.30 (±0.14) and 0.09 (±0.04) after 24 h and 48 h, respectively. Moreover, co-delivery of SN38and Snail specific siRNA in an appropriate nanocerrier (chitosan nanoparticles) significantly reduced the viability and proliferation rate of the PC-3 cells. Conclusion: In conclusion, ChNPs encapsulating SN38 and Snail-specific siRNA may represent huge Potential as an effective anti-cancer drug delivery system for the treatment of metastatic prostate cancer.

P.D3.04 Novel approaches to vaccinology - Part 4

P.D3.04.01

Transcriptome profiling in blood before and after hepatitis B vaccination shows significant differences in gene expression between responders and non-responders

***E. Bartholomeus**[1,2], N. De Neuter[3,2,4], P. Meysman[3,2,4], A. Suls[1,2], N. Keersmaekers[5,2], G. Elias[6,2], H. Jansens[7], N. Hens[5,2,8], E. Smits[6,9,10], V. Van Tendeloo[6,2], P. Beutels[5,2], P. Vandamme[11,2], B. Ogunjimi[5,2,12], K. Laukens[3,2,4], G. Mortier[1,2];*
[1]Center of Medical Genetics, University of Antwerp, Edegem, Belgium, [2]Antwerp Unit for Data Analysis and Computation in Immunology and Sequencing, University of Antwerp, Antwerp, Belgium, [3]Adrem Data Lab, University of Antwerp, Antwerp, Belgium, [4]Biomedical Informatics Research Network Antwerp (biomina), University of Antwerp, Antwerp, Belgium, [5]Centre for Health Economics Research & Modeling Infectious Diseases (CHERMID), Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerp, Belgium, [6]Laboratory of Experimental Hematology (LEH), Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerp, Belgium, [7]Department of Laboratory Medicine, Antwerp University Hospital, Edegem, Belgium, [8]Interuniversity Institute for Biostatistics and Statistical Bioinformatics, Hasselt University, Diepenbeek, Belgium, [9]Center for Cell Therapy and Regenerative Medicine, Antwerp University Hospital, Edegem, Belgium, [10]Center for Oncological Research Antwerp, University of Antwerp, Antwerp, Belgium, [11]Centre for the Evaluation of Vaccination (CEV), Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerp, Belgium, [12]Department of Paediatrics, Antwerp University Hospital, Edegem, Belgium.

Introduction: As the hepatitis B virus is widely spread and responsible for considerable morbidity and mortality, WHO recommends vaccination from infancy to reduce acute infection and chronic carriers. However, current subunit vaccines are not 100% efficacious and leave 5-10% of recipients unprotected.
Methods: To evaluate immune responses after Engerix-B vaccination, we determined, using 3' mRNA-sequencing, whole blood early gene expression signatures before, at day 3 and day 7 after the first dose and correlated this with the resulting antibody titer after two vaccine doses.
Results: Our results indicate that immune related genes are differentially expressed in the responders mostly at day 3 and in the non-responders mostly at day 7. The most remarkable difference between responders and non-responders were the differentially expressed genes at day 0 before vaccination. The granulin precursor gene (GRN) was significantly downregulated in responders while upregulated in non-responders at day 0. Furthermore, absolute granulocytes numbers were significantly higher in non-responders at day 0.
Conclusion: The non-responders already showed an activated state of the immune system before vaccination. Furthermore, after vaccination, they exhibited a delayed and partial immune response in comparison to the responders. Our data may indicate that the baseline and untriggered immune system can influence the response upon hepatitis B vaccination.

P.D3.04.02

An APC-targeted PfRH5-containing DNA vaccine induce protective immune responses against *Plasmodium falciparum*

***L. Bjerkan**[1], R. Braathen[1], G. Ram Visweswaran[1], G. Grødeland[1], A. Gudjonsson[1], G. Labbe[2], S. Draper[2], B. Bogen[1];*
[1]Institute of Clinical Medicine, Oslo, Norway, [2]Jenner Institute, Oxford, United Kingdom.

The development of an effective, safe and deployable malaria vaccine remains an urgent priority for improving global public health. Targeted delivery of antigen to antigen presenting cells (APC) is an efficient way to increase specific immune responses. Here, we present a DNA vaccine that targets *Plasmodium falciparum* RH5 (PfRH5) antigen to major histocompatibility complex (MHC) class II molecules expressing APC.
The PfRH5 antigen was cloned into bivalent homodimeric Ig-based molecules (vaccibodies) bearing anti MHC class II scFv. We showed that this vaccine strategy induced high titers of PfRH5-specific antibodies in BALB/c mice that efficiently inhibited the growth of the *Plasmodium falciparum* 3D7 clone *in vitro*. Furthermore, the APC-targeted PfRH5 vaccine efficiently induced rapid peptide specific IFN-γ T cell responses in mice. To prepare for translation into human vaccination, we constructed a DNA vaccine that targeted HLA class II (HLA-II) molecules which cross-react with MHC- class II molecules in several species of larger mammals, including humans. We demonstrated induction of PfRH5-specific antibody responses in vaccinated pigs and that this APC targeted DNA vaccine showed no toxicity.
In conclusion, these results reveal a novel vaccination strategy for development of future vaccines against malaria.

P.D3.04.03

Cross-reactivity of anti-dengue human monoclonal antibodies with zika virus (ZIKV)

***K. Boonha**[1], S. Benjathummarak[1], P. Pitaksajjakul[1,2], P. Ramasoota[1,2];*
[1]Center of Excellence for Antibody Research (CEAR), Faculty of Tropical Medicine, Mahidol University, BANGKOK, Thailand, [2]Department of Social and Environmental Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

ZIKV is a mosquito-borne belonging to similar flavivirus family with Dengue virus (DENV). ZIKV and DENV serotype 2 share 54% sequence identity of full envelope protein and 100% identity of fusion loop protein. The correlation and homology of ZIKV and DENV may play a role in the pre-existing immunity to DENV. Eventhough, anti-fusion loop antibodies was considered as the major populations found in human immune response, and showed protective activity, the information of antibodies that found in ZIKV infected patients is still limited.

We firstly screened the binding of our 20 human monoclonal antibodies (huMAbs) that showed strong neutralizing activity against all 4 DENV serotypes with ZIKV by IFA. These huMAbs were characterized their binding regions on domain II of E protein and showed cross-reactivity with other flavivirus like Japanese encephalitis virus (JEV) and some showed strong neutralizing activity against JEV. It was shown that 19 of 20 huMAbs shown strong binding activity with ZIKV, as expected by their highly conserved of fusion loop region among the two species. Cross-reactive anti-DENV huMAbs with ZIKV were tested for neutralizing activity using focus reduction neutralization test in Vero cell. It was shown that most of huMAbs that targeted to envelop DII showed low neutralizing activity. Different with DENV, it was hypothesized that our anti-fusion loop huMAbs might not be the target epitope of ZIKV neutralization and protective activity in ZIKA infected patients. Further study of antibody inhibition ELISA using ZIKV infected serum with several kind antibodies specific to different epitope might showed the real situation of the antibody response of ZIKV infection.

P.D3.04.04

Measles vaccination before 9 months of age results in reduced antibody functionality long-term

I. Brinkman[1], *A. Butler*[2], *J. de Wit*[1], *G. Smits*[1], *H. ten Hulscher*[1], *R. Jongerius*[1], *F. van der Klis*[1], *N. Rots*[1], *D. van Baarle*[1], *G. Alter*[2], *R. van Binnendijk*[1];
[1]*National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands,* [2]*Ragon Institute of MGH, MIT and Harvard, Cambridge, United States.*

Background Measles is one of the most infectious viruses, but effective vaccination significantly reduces the amount of measles cases. However, as maternal antibodies are no longer present around 6 months of age, young infants are at risk until they receive their primary vaccination between 12-15 months of age. This provides a strong incentive to reduce the age of measles vaccination, but the long-term immunological consequences are largely unknown.
Methods Children who received first measles vaccination between 6-8 months or 9-12 months and a second dose at 14 months of age were compared with a control group who only received one dose at 14 months of age. Effectiveness of measles-specific antibodies was determined at 14 months and 1 and 3 years later by systems serology.
Results The majority of children that received a first measles vaccination between 6-12 months induced a significant measles-specific antibody response. However, when vaccinated before 9 months of age measles-neutralizing antibody concentrations were lower, and induction of phagocytosis by monocytes and neutrophils as well as complement deposition were affected compared with children who received a first dose after 9 months of age. The reduced effectiveness of antibodies increased over time after vaccination.
Conclusions Early measles vaccination provides short-term protection in the majority of children, but children vaccinated before 9 months of age have a less functional antibodies compared with children vaccinated at a later age. Eventually, this may result in an increasing number of children susceptible to measles long-term.

P.D3.04.05

Transcriptomic analysis of the blood immune response to the rVSV-ZEBOV Ebola vaccine

F. Santoro[1], ***A. Donato***[1], *A. Gerlini*[2], *S. Lucchesi*[1], *S. Sorgi*[1], *M. C. Haks*[3], *A. Huttner*[4], *A. M. Harandi*[5], *T. P. Monath*[6], *T. H. Ottenhoff*[3], *C. Siegrist*[4], *G. Pozzi*[1], *D. Medaglini*[1], *the VSV-EBOVAC consortium, the VSV-EBOPLUS consortium;*
[1]*University of Siena, Siena, Italy,* [2]*Microbiotec srl, Siena, Italy,* [3]*Leiden University Medical Center, Leiden, Netherlands,* [4]*University of Geneva, Geneva, Switzerland,* [5]*University of Gotheborg, Gotheborg, Sweden,* [6]*Crozet BioPharma LLC, Devens, United States.*

rVSV-ZEBOV is a live-attenuated recombinant vesicular stomatitis virus vaccine expressing Ebolavirus glycoprotein G and is the only Ebola vaccine with demonstrated clinical efficacy. Here we studied the blood transcriptomic response upon injection of a single dose of vaccine. Whole blood RNA from 64 healthy volunteers, 51 injected either with 10^7 or $5x10^7$ PFU of rVSV-ZEBOV and 13 with placebo, collected at different time points after vaccination, was analyzed by targeted transcriptome sequencing. At each time point, differentially expressed genes (DEGs) were identified with edgeR, ranked by FDR, and used to find biological signatures assessing the activation of 346 blood transcription modules. Between baseline and day 1 after vaccination, 5,469 DEGs were detected. This number decreased over time: at day 35 only 10 DEGs were detected. Functional analysis identified 135 different modules affected by vaccination. Innate immunity pathways were activated from day 1 to day 14. At days 2 and 3, neutrophil modules were downregulated and complement-related modules upregulated. T-cell and cell-cycle associated modules were upregulated at days 7 and 14, while at day 28 no modules remained activated. Correlation analyses of gene expression with ZEBOV glycoprotein-specific antibody titers identified 15 strongly correlated genes at day 14 after vaccination (absolute Spearman's Rho>0.5, p<0.001).Vaccination with rVSV-ZEBOV induced a strong and durable modulation of innate response associated genes. An algorithm correlating with antibody titers one year after vaccination was developed based on the expression levels of 15 genes. Study supported by IMI2-JU Ebola+ program under VSV-EBOVAC [grant 115842] and VSV-EBOPLUS [grant 116068] projects.

P.D3.04.06

Potential impact of maternal vaccination on life-threatening respiratory syncytial virus infection during infancy

N. M. Scheltema[1], *X. M. Kavelaars*[2], *K. Thorburn*[3], *M. P. Hennus*[4], *J. B. van Woensel*[5], *C. K. van der Ent*[6], *J. A. Borghans*[2], *L. L. Bont*[1], ***J. Drylewicz***[2];
[1]*University Medical Center Utrecht, Department of Paediatric infectious diseases and immunology, Utrecht, Netherlands,* [2]*University Medical Center Utrecht, Laboratory of Translational Immunology, Utrecht, Netherlands,* [3]*Department of Paediatric Intensive Care, Alder Hey Children's Hospital, Liverpool, United Kingdom,* [4]*University Medical Center Utrecht, Department of Paediatric Intensive Care, Utrecht, Netherlands,* [5]*Academic Medical Centre Amsterdam, Department of Paediatric Intensive Care, Amsterdam, Netherlands,* [6]*University Medical Center Utrecht, Department of Paediatric pulmonology, Utrecht, Netherlands.*

Respiratory syncytial virus (RSV) infection is an important cause of infant mortality. Here, we estimated the potential impact of maternal vaccination against RSV on life-threatening RSV infection in infants. We developed a mathematical model of maternal vaccine-induced antibody dynamics taking into account transplacental antibody transfer and antibody decline after birth. The model was applied to data from two cohorts of children younger than 12 months with RSV-related paediatric intensive care unit (PICU) admission in the United Kingdom (n = 370) and the Netherlands (n = 167), and a cohort of 211 children younger than 12 months with RSV-related in-hospital death from 20 countries worldwide. For each cohort, we predicted the percentage of children with life-threatening RSV infection potentially prevented by maternal vaccination. Our model predicted that, depending on vaccine efficiency, maternal vaccination at 30 weeks gestational age could have prevented 62-75% of RSV-related PICU admissions in the United Kingdom and 76-87% in the Netherlands. For the global mortality cohort, the model predicted that maternal vaccination could have prevented 29-48% of RSV-related deaths. Preterm children and children with comorbidities were predicted to benefit less than (healthy) term children. In conclusion, maternal vaccination against RSV may substantially decrease life-threatening RSV infections in infants.

P.D3.04.07

High-dimensional profiling of early immune events following acellular pertussis booster vaccination

J. Gillard[1,2], *P. Brazda*[3], *N. Atlasy*[3], *M. Suffiotti*[4], *M. I. de Jonge*[1,2], *E. Simonetti*[1,2], *E. M. Janssen-Megens*[3], *C. Teodosio*[5], *R. de Groot*[1,2], *G. Pantaleo*[4], *G. Berbers*[6], *C. Fenwick*[4], *H. Stunnenberg*[3], *D. Diavatopoulos*[1,2];
[1]*Section Pediatric Infectious Diseases, Laboratory of Medical Immunology, Radboud Institute for Molecular Life sciences, Nijmegen, Netherlands,* [2]*Radboud Center for Infectious Diseases, Radboudumc, Nijmegen, Netherlands,* [3]*Department of Molecular Biology, Radboud University, Faculty of Science, Nijmegen, Netherlands,* [4]*Swiss Vaccine Institute, Lausanne, Switzerland,* [5]*Leiden University Medical Center, Department of immunohematology and blood transfusion, Leiden, Netherlands,* [6]*National Institute for Public Health and Environment (RIVM), Center for Infectious Disease Control (Cib), Biltoven, Netherlands.*

Many countries continue to experience pertussis epidemics in spite of widespread vaccination. Moreover, increasing disease incidence has been observed in completely vaccinated children, adolescents and adults. It is thought that the first vaccine dose given during infancy programs long-term immunity to pertussis, with acellular (aP) and whole cell pertussis (wP) vaccines inducing distinct immune profiles. Thus the objective of this study is to apply systems vaccinology to study the early innate immune response to aP booster vaccination in young adolescents primed with either aP or wP vaccines during infancy. We characterized early immune events before, and 24 hours after booster vaccination using complementary tools. Deep phenotyping of circulating immune cells was performed with a specialized mass cytometry (CyTOF) panel for innate responses. In parallel, flow cytometry was used to further characterize the immune response and to obtain single innate immune cells by index sorting, thereby bridging our cytometry dataset and downstream gene expression analysis through single-cell RNA sequencing (scRNAseq). We found that both cytometry datasets display high concordance, including shifts in granulocyte and myeloid populations post-vaccination. scRNAseq and correlation analysis of early innate immunity with long-term pertussis-specific immunity is ongoing. This study provides novel insights into the molecular mechanisms underlying the immune response to aP booster vaccination and provides an important framework for the development of new pertussis booster vaccines.

P.D3.04.08

MHC class II targeted DNA vaccine is most efficient in the induction of protective antibodies against influenza

D. M. Hinke, *H. C. Spång, E. Fossum, G. Grødeland, B. Bogen, R. Braathen;*
K.G. Jebsen Centre for Influenza Vaccine Research, Oslo, Norway.

Most successful vaccines owe their efficacy to induction of protective antibodies. Vaccine formats that induce high and long-lasting antibody responses will be highly interesting. One approach, APC-targeted DNA vaccination, explores that transfected cells secrete fusion proteins with targeting units specific for surface molecules on APC. This targeting of antigen to the APC increases delivery of antigen, resulting in improved immune responses. We have benchmarked several different targeting units in their ability to influence the magnitude of antibody responses against hemagglutinin (HA) from influenza A virus.

We created DNA plasmid vaccines that encode bivalent homodimeric Ig-based molecules (vaccibodies) which express two HA antigens fused to two APC targeting units. The results showed that targeting of Xcr1$^+$ type 1 conventional dendritic cells, using Xcl1, MIP-1α, αCD40 and αDEC205 targeting units, preferentially induced IgG2a responses. Simultaneous targeting of several dendritic cell subtypes in addition induced IgG1 responses, as shown for αCD11c, αMHCII, Flt-3L and FliC targeting units. IgG1 responses occurred early after immunization but declined relatively rapid over time. IgG2a responses appeared later but lasted longer (>252 days). The overall antibody induction in BALB/c mice depended on the targeting units in the following order: αMHCII>αCD11c>αCD40>Xcl-1=MIP-1α>FliC>GM-CSF>Flt-3L>αDEC205. MHC class II targeted DNA vaccines elicited complete short- and long-term protection against influenza virus. Other antigens and T cell assays will be included to confirm the role of the various targeting units in the magnitude of the responses.
In conclusion, targeting a wide range of APC with MHC class II targeting unit induces protective antibodies against influenza.

P.D3.04.09

Uncovering the epitopes underlying the induction of varying adaptive immune responses by different *Mycobacterium tuberculosis* lineages

C. Magalhães[1,2]*, I. Comas*[3,4]*, M. Saraiva*[5,6]*, N. S. Osório*[1,2]*;*
[1]*Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal,* [2]*ICVS/3B's - PT Government Associate Laboratory, Braga, Portugal,* [3]*CIBER en Epidemiología y Salud Pública, Valencia, Spain,* [4]*Instituto de Biomedicina, IBV-CSIC, Valencia, Spain,* [5]*i3S - Instituto de Investigação e Inovação em Saúde, Porto, Portugal,* [6]*IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal.*

Tuberculosis (TB) is the deadliest infectious disease in the history of humankind and remains vastly uncontrolled. Active TB results from infection with distinct genetic lineages of the *Mycobacterium tuberculosis* complex. Interestingly, a strong geographic association between TB cases and specific lineages exists, which is disrupted in the context of HIV-1 co-infection. This fact highlights the relevance of CD4$^+$ T cell-driven immune responses in the interaction between different human/pathogen populations and TB outcome. *M. tuberculosis* lineage-specific epitope diversity might thus alter the type and level of CD4$^+$ T cell responses generated during infection. Despite the importance of this topic, host-pathogen molecular characteristics influencing immune synapse in TB are not sufficiently studied.
We have developed a genome-wide immunoinformatics approach to identify T cell epitopes that are influenced by the presence of *M. tuberculosis* lineage-specific polymorphisms. Importantly, it was possible to find significant associations between HLA-binding predictions for a given lineage and the frequency of the HLAs in the human populations with more TB caused by that lineage. Some mutant epitopes were also inferred to have been selected over time by distinct computational molecular evolution methodologies.
Overall, this study suggests that specific *M. tuberculosis* lineage-restricted polymorphisms have been fixed during parallel evolution with the host due to CD4$^+$ T cell pressure. The identification and extensive characterization of varying *M. tuberculosis* epitopes might be of great relevance for the development of more effective TB vaccination and diagnostics strategies.

P.D3.04.10

Immunisation with a conserved rhinovirus capsid protein induces antibodies that bind a variable neutralising epitope

S. Narean[1]*, C. Nunn*[1]*, N. Glanville*[2]*, S. Johnston*[2]*,* ***G. McLean***[1,2]*;*
[1]*Cellular and Molecular Immunology Research Centre, London, United Kingdom,* [2]*National Heart and Lung Institute, Imperial College London, London, United Kingdom.*

Introduction: Human rhinovirus (RV) infections are the principle cause of common colds and precipitate asthma and chronic obstructive pulmonary disease exacerbations. Currently there is no vaccine for RV which is largely due to the existence of ~150 serotypes/strains. We demonstrated previously that immunising mice with highly conserved VP4 and VP2 regions of the RV polyprotein (RV16 VP0) generated broadly cross-reactive protective immunity to RV *in vivo*. This study investigated and mapped the epitopes of RV16 VP0 that are targets for neutralising polyclonal antibody responses. **Materials and Methods:** Serum samples from VP0 immunisation and RV challenge studies in mice were used to determine IgG recognition sites by ELISA and *in vitro* RV neutralisation assay. Peptide pools and individual peptides spanning the immunogen sequence (RV16 VP0) were assessed for IgG binding sites to identify neutralising epitopes. **Results:** Eight peptide pools containing 15-mer peptides spanning the RV16 VP0 sequence were assessed for binding by antisera obtained from RV16 VP0 immunised and RV challenged mice.
We found that peptide pools covering the C-terminus of VP4, N-terminus of VP2 and the neutralising NIm-II loop within VP2 were bound by serum IgG but not by serum IgA. The NIm-II loop peptide pool blocked IgG binding to the immunogen RV16 VP0 but was unable to inhibit IgG neutralisation of RV *in vitro*. **Conclusions:** We have identified immunodominant epitopes of RV vaccine candidate RV16 VP0. Strong polyclonal IgG binding antibodies were observed that target a key neutralising epitope that is highly variable amongst RV serotypes.

P.D3.04.11

Searching for novel vaccine candidates against *Echinococcus granulosus* combining proteomic and bioinformatic explorations of tegumental antigens.

S. Miles[1]*, M. Portela*[2]*, M. Cyrklaff*[3]*, M. Ancarola*[4]*, F. Frischknecht*[3]*, R. Durán*[2]*, S. Dematteis*[1]*, G. Mourglia-Ettlin*[1]*;*
[1]*Immunology Lab - DEPBIO - UdelaR, Montevideo, Uruguay,* [2]*Institut Pasteur de Montevideo, Montevideo, Uruguay,* [3]*Integrative Parasitology, Center for Infectious Diseases, Heidelberg University, Heidelberg, Germany,* [4]*Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPaM, UBA-CONICET), Facultad de Medicina, Buenos Aires, Argentina.*

Echinococcus granulosus is the helminth parasite responsible for cystic echinococcosis (CE), an important worldwide-distributed zoonotic disease. Development of new vaccines against CE might have great health and economic benefits. Here, we described an innovative vaccine design scheme starting from a tegumental antigens-enriched fraction derived from protoscoleces -named PSEx- already known to induce protection against CE. First, we characterized by mass spectrometry its protein composition. Then, by means of Gene Ontology analyses, we studied the potential biological processes, molecular functions and cellular localizations associated with identified PSEx components. After that, antigenicity predictions and determination of conservancy degree against other organisms were determined. Thus, 10 proteins -identified here for the first time- were proposed as novel vaccine candidates. Furthermore, linear B-cell epitopes free of post-translational modifications were predicted in the whole PSEx proteome through co-localization of *in silico* predicted epitopes within peptide fragments identified by MALDI-TOF/TOF. Resulting peptides were termed "clean linear B-cell epitopes", and through BLASTp scanning against all non-helminth proteins, those with 100% identity against any other protein were discarded. Then, secondary structure was predicted for the peptides -free and within their parental proteins- and those showing highly similar secondary structure in both cases were selected. Potentially toxic and/or allergenic peptides were discarded. Finally, selected clean linear B-cell epitopes were mapped within their corresponding 3D-modelled parental protein to assess their possible antibody accessibilities, resulting in 14 putative peptide vaccine candidates. At the end, we proposed 10 novel proteins and 14 peptides that deserve further testing as vaccine candidates against CE.

P.D3.04.12

Characterization of the porcine MHC I in the Goettingen minipig

B. von Silva-Tarouca*, M. Wu, D. Zehn;*
School of Life Sciences Weihenstephan, Freising, Germany.

Mice are widely used as an experimental system to explore all aspects of immunity. However, mice and humans have fundamental differences, as demonstrated by the many cases of unsuccessful translation of results from mice to human. Given the strong need to better evaluate therapeutic interventions that are based on biological substances, there is a high demand for well-characterized and robust alternative testing systems.
We propose that large animal-based models, such as minipigs, constitute an important alternative or complementary test system. One key limitation is that the murine immune system is much better characterized than the immune system in pigs, especially antigen-specific T cell responses. Therefore, we aim to advance the studies of adoptive immunity in pigs using a two-step strategy, beginning with the identification of frequently occurring MHC-alleles and followed by establishing a test system to screen peptide-restricted antigens by these MHC-alleles.
Using pooled next generation sequencing we have subtyped the SLA I molecules of 53 minipigs. To further improve the significance of our investigation we also included 25 samples from Landrace pigs and 65 samples from pigs raised on conventional farms. Contrary to the expectations, a high number of animals were homozygous for one or more SLA I-chains. The obtained data enabled us to identify alleles that are more frequent and we are now developing a MHC-peptide binding and epitope-prediction-assay based on these findings, which will allow us to investigate different aspects of infections like the PRRS-virus with valuable insights for new vaccines, and mechanisms of anti-tumor immunity.

P.D3.04.13

Comparative Immunogenicity protective efficacy of equivalent bivalent Generalized Modules for Membrane Antigens (GMMA) glycoconjugate vaccines against nontyphoidal Salmonella

F. Necchi[1], *R. Alfini*[1], *L. Lanzilao*[1], *O. Rossi*[1], *A. Negrea*[2], *S. Clare*[3], *P. Mastroeni*[4], *A. Saul*[1], *C. A. MacLennan*[5], *S. Rondini*[1], *F. Micoli*[1];
[1]*GSK Vaccines Institute for Global Health S.r.l. (GVGH), Siena, Italy,* [2]*Employee at Novartis Vaccines Institute for Global Health (now GVGH) at the time of the study, Siena, Italy,* [3]*Wellcome Trust Sanger Institute, Cambridge, United Kingdom,* [4]*Department Of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom,* [5]*Jenner Institute, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom.*

Invasive nontyphoidal *Salmonella* (iNTS) disease is a leading cause of death and morbidity in Africa. The most common pathogens are *Salmonella enterica* serovars Typhimurium and Enteritidis. The O-antigen portion of their lipopolysaccharide is a target of protective immunity, but no vaccines are currently available, or in clinical trials. Here we investigate the use of Generalized Modules for Membrane Antigens (GMMA) as delivery system for *S.* Typhimurium and *S.* Enteritidis O-antigen, and compare this technology to the more traditional glycoconjugation approach. GMMA are outer membrane vesicles released from genetically engineered Gram-negative bacteria, carrying deletion of the *tolR* gene or analogue to enhance their production. *Salmonella tolR*$^-$ strains were generated for GMMA production. O-antigens were extracted from corresponding wild-type bacteria and conjugated to CRM_{197}. Purified GMMA and conjugates were characterized, showing similar O-antigen structural characteristics. When compared in mice, GMMA gave higher anti-O-antigen IgG titers compared to conjugate, in the absence of Alhydrogel. With Alhydrogel, antibody levels were similar. Antibody isotype profile was also investigated, showing a diverse Ig subclass repertoire induced by GMMA, with greater serum bactericidal activity compared to that induced by the glycoconjugate. In an *in vivo* mouse infection model, bacterial colonization was reduced upon infection with *Salmonella*, with *S*. Typhimurium counts lower with GMMA and with *S.* Enteritidis burden similar with both vaccines. Overall, simplicity of manufacturing process and low costs of production, coupled with encouraging immunogenicity data, make GMMA an attractive strategy for the development of a nontyphoidal *Salmonella* vaccine compared with established glycoconjugate technology.

P.D3.04.14

Assessment of antigen-specific B and T cell responses following TdaP vaccination in adults

F. Schiavetti[1], *E. Faenzi*[1], *F. Buricchi*[1], *E. Borgogni*[1], *M. Bardelli*[1], *F. Spensieri*[1], *A. Seubert*[1], *M. Pizza*[1], *G. Leroux-Roels*[2], *S. Bertholet*[1], *O. Finco*[1], *G. Del Giudice*[1];
[1]*GSK, Siena, Italy,* [2]*Centre for Vaccinology, Ghent University and University Hospital, Ghent, Belgium.*

Pertussis remains an important cause of infant mortality despite global infant vaccination programs. The recent resurgence of *Bordetella pertussis* infections worldwide is calling for new vaccines inducing longer-lasting protection. The magnitude and persistence of pertussis-specific immunity was explored during a clinical study[1] (ClinicalTrials.gov NCT01529645) evaluating the safety and immunogenicity of different doses of booster vaccines of acellular pertussis (aP) in combination with diphtheria and tetanus antigens (TdaP) in adults. Participants received TdaP formulations, containing either the genetically or chemically detoxified pertussis toxin (PT) in combination with the filamentous hemagglutinin (FHA) and pertactin (PRN) antigens. Frequencies of antigen-specific plasmablasts (PB, at day [D] 8 after vaccination), $CD4^+$ T cells (at baseline, D8 and D30 after vaccination) and Memory B Cells (MBC) (at baseline, on D30 and D365 after vaccination) were assessed. All vaccine formulations expanded antigen-specific IgG^+ MBC and PB against FHA, PRN and PT. Remarkably, genetically detoxified PT induced higher frequencies of MBC than chemically detoxified PT, that remained higher one year after vaccination. Lack of IgM^+ PB and low frequencies of IgM^+ MBC in any of the vaccination groups indicate pre-existing immunity to pertussis antigens. Frequencies of PRN, FHA and PT-specific $CD4^+$ T cells increased at D8 and one month post-vaccination without major differences between groups. Overall the data reported here support the evidence of a stronger propensity of genetically detoxified PT to induce immunological responses which might prove promising for next generation pertussis vaccines.
[1]Leroux-Roels G et al, Hum Vaccin Immunother 2018;14(1):45–58
Funding: GlaxoSmithKline Biologicals SA

P.D3.04.15

Ex vivo Generation and Single-Cell Analysis of Human Monoclonal Antibodies from Dengue Virus Infected Patients

P. Sharma[1], *H. K. Panda*[1], *M. Singla*[2], *E. S. Reddy*[1], *R. Shukla*[3], *N. Khanna*[3], *K. Nayak*[1], *K. Dixit*[1], *R. Lodha*[2], *G. Medigeshi*[4], *R. Ahmed*[5], *A. Chandele*[1], *M. K. Kaja*[1,6];
[1]*ICGEB Emory Vaccine Centre,International Centre for Genetic Engineering and Biotechnology, New Delhi, NEW DELHI, India,* [2]*All India Institute of Medical Science, New Delhi-110029, NEW DELHI, India,* [3]*Recombinant Gene Products-International Centre for Genetic Engineering and Biotechnology, New Delhi, NEW DELHI, India,* [4]*Translational Health Science and Technology Institute, Faridabad-121004, NEW DELHI, India,* [5]*Emory Vaccine Center, Atlanta, GA, Atlanta, United States,* [6]*Department of Paediatrics-Emory Vaccine Center, Atlanta, GA, United States.*

Antibodies have been implicated in both protection and pathology of dengue virus infections. However, much of this data is gathered from serum/plasma responses that is a cumulative of historical and ongoing infection. To precisely understand the role of antibodies with respect to the ongoing dengue virus infection, we employed the cutting edge approach of generating of human monoclonal antibodies from individual plasmablasts from peripheral blood of dengue patients that allows us to probe for answers at a single cell level. This method involves *ex vivo* single cell sorting of plasmablasts from peripheral blood of characterised dengue infected patient followed by single cell molecular cloning of immunoglobulin heavy- and light- variable regions into expression vectors containing the defined constant region followed by transient co-transfection of HEK 293A cells with the heavy and light chain expression vectors made from genes arising from the same cell. Thus far, using this powerful technology, for the first time in India, we have made 73 number of human monoclonals, of which 36 are specific to dengue and 12 neutralize dengue at various concentrations. Interestingly, all the dengue neutralizing antibodies were envelope specific and recognised the highly conserved fusion loop of the dengue virus envelope. Together, with the ongoing comprehensive analysis of the B cell repertoire and somatic hypermutations, these studies provide a detailed understanding of the dengue-specific plasmablast cell response at a single cell level and create a platform for testing these antibodies for basic research, diagnostic, prophylactic and as well as therapeutic applications.

P.D3.04.16

Salmonella enterica **serovar Typhimurium SiiE interferes with the persistence of IgG-secreting plasma cells in laminin â1$^+$ bone marrow survival niches and prevents efficient vaccination**

Y. Yamasaki[1], *C. Männe*[1], *M. Mursell*[1], *F. Hiepe*[1], *S. H. Kaufmann*[2], *A. Radbruch*[1], ***K. Tokoyoda***[1];
[1]*Deutsches Rheuma-Forschingszentrum Berlin, Berlin, Germany,* [2]*Max Planck Institute for Infection Biology, Berlin, Germany.*

Invasive non-typhoidal *Salmonella* (iNTS) disease induced commonly by *Salmonella enterica* serovar Typhimurium was estimated to cause 3.4 million illnesses and 680,000 deaths (the fatality rate at ~20%) per year. No vaccine against non-typhoidal *Salmonella* is currently available, albeit multiple drug-resistant non-typhoidal *Salmonella* is already highly prevalent. We here report that the protein SiiE of *S.* Typhimurium is required and sufficient to prevent an efficient humoral immune response, selectively reducing the numbers of IgG-secreting plasma cells in the bone marrow (BM). Attenuated SiiE-deficient *S.* Typhimurium induces high and lasting titers of specific and protective IgG, and qualifies as the first efficient vaccine for the serotype. An SiiE-derived peptide with homology to laminin β1 is sufficient to ablate IgG-secreting plasma cells from the BM, identifying laminin β1 as a novel and essential component of niches for IgG-secreting plasma cells in the BM, and furthermore qualifies as a unique therapeutic option to ablate selectively IgG-secreting plasma cells in autoimmune diseases and multiple myeloma.

P.D3.04.17

IgA targeting HIV-1 envelope gp41 triggers antibody-dependent cell cytotoxicity cross-clade and cooperates with gp41 specific IgG to increase cell lysis

D. Tudor[1,2,3], *M. Duchemin*[1,2], *M. Khamassi*[1], *L. Xu*[1], *M. Bomsel*[1];
[1]*Laboratory of Mucosal Entry of HIV-1 and Mucosal Immunity, Department of Infection, Immunity and Inflammation, Cochin Institute, CNRS UMR 8104, Paris, France,* [2]*INSERM U1016, Paris, France,* [3]*Université Paris Descartes, Sorbonne Paris Cité, Paris, France.*

Introduction: Although raising broadly neutralizing antibodies (bNAbs) are the main goal of vaccination against HIV-1, growing evidence show that protecting antibodies could also control viral infection by Fc-mediated antiviral activity, such as antibody dependent cellular cytotoxicity (ADCC). Until now, mainly gp120 and gp41 HIV-1 envelope-specific IgGs have been shown to mediate ADCC whereas the ADCC potential of HIV-1 envelope-specific IgA remains elusive, despite the prevalence of IgA at mucosal level, the main portal entry for HIV-1. **Methods:** The capacity of HIV-1 envelope-specific IgA to induce Fcα-mediated ADCC was evaluated by flow cytometry using 2F5-IgA genetically engineered from the broadly neutralizing gp41-specific 2F5-IgG we have previously reported to induce ADCC. Effector cells were primary monocytes and target cells were $CD4^+$T lymphocytes either infected with the main HIV-1 subtypes, namely A, B or C, or coated with the P1-A, -B, and -C peptides covering the MPR region of gp41 from subtype A, B and C, respectively. **Results:** We show here that 2F5-IgA, targeting subtype A and B, but not C gp41, engages FcαRI (CD89), expressed on human monocytes, to induce the lysis of subtype A and B, but not C, HIV-1-infected cells and of P1-A and -B, but not -C, coated cells by ADCC. Furthermore, the 2F5-IgA cooperates with 2F5-IgG as well as with the bNAb gp41-specific 10E8-IgG to enhance target-cells lysis by ADCC. **Conclusion:** These results indicate that inducing IgA by vaccination, especially those targeting gp41, together with IgG could strength HIV-1 mucosal protection or reservoir eradication worldwide by mediating ADCC.

P.D3.04.18

Incidence and serotype distribution of pneumococcal pneumonia in Bangladeshi children under 5 years of age

S. Vestjens[1], *T. Lalmahomed*[2], *B. Meek*[1], *B. de Jong*[1], *D. Goswami*[3], *S. van Mens*[4], *B. Vlaminckx*[1], *D. Ahmed*[3], *H. Endtz*[5], *A. Brooks*[3], *G. Rijkers*[1,2];
[1]*St Antonius hospital, Nieuwegein, Netherlands,* [2]*University College Roosevelt, Middelburg, Netherlands,* [3]*ICDDR,B, Dhaka, Bangladesh,* [4]*MUMC+, Maastricht, Netherlands,* [5]*Erasmus university, Rotterdam, Netherlands.*

Introduction. Pneumonia is the leading cause of mortality in children under 5 years of age in developing countries, most often caused by *Streptococcus pneumoniae*. Pneumococcal conjugate vaccines (PCVs) for infants have been introduced in many national immunisation programmes. This includes the 10-valent PCV in Bangladesh in 2015, even though no data is available regarding pneumococcal serotype incidence. Our aim was to identify pneumococcal serotypes causing pneumonia in children under 5 in Bangladesh. **Methods**. From 1533 children aged 0 to 59 months with upper respiratory infection or pneumonia, serum samples were obtained at diagnosis and another ≥14 days later. Pneumococcus serotype-specific antibody responses were measured using a multiplex immunoassay panel of 25 serotypes (1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12A, 12F, 14, 15B, 18C, 19A, 19F, 20, 22F, 23F, 33F and 45). Calculating the fold increase of the antibody response between the second and first serum sample allowed to determine prevalence of pneumococcus-mediated pneumonia and the causative serotype. **Results**. Serotype-specific pneumococcal antibody responses were detected in 31% of the children. The 10 most frequently identified serotypes were 11A, 22F, 3, 2, 19F, 45, 15B, 19A, 33F, and 9N. **Conclusions** Our findings demonstrate that the 10-valent PCV used in Bangladesh yields low coverage of the most prevalent disease-causing serotypes. Although the identified serotypes may not all cause invasive pneumococcal disease, vaccines will be more effective if the prevalence serotypes is taken into account. Future vaccine combinations may improve coverage for the India/Bangladesh region.

P.D3.04.19

Detection of YF-17D viral RNA in human antigen-presenting cells by RNA flow cytometry

E. Winheim[1], *L. Habenicht*[1,2], *K. Eisenaecher*[1], *M. K. Scheck*[3,4], *M. Pritsch*[1,5], *J. Ahlfeld*[3,4], *S. Rothenfusser*[3,4], *A. B. Krug*[1];
[1]*Institute for Immunology, Biomedical Centrum, Ludwig Maximilian University of Munich, Planegg-Martinsried, Germany,* [2]*Klinik und Poliklinik für Innere Medizin II, Klinikum rechts der Isar, Technische Universitaet Muenchen, Munich, Germany,* [3]*Division of Clinical Pharmacology, Department of Medicine IV, University Hospital, Ludwig Maximilian University of Munich, Munich, Germany,* [4]*Einheit fuer Klinische Pharmakologie (EKliP), Helmholtz Zentrum Munich, Neuherberg, Germany,* [5]*Division of Infectious Diseases and Tropical Medicine, Medical Center of the University of Munich, Munich, Germany.*

Flaviviruses, a family of positive-strand RNA viruses, including yellow fever (YF), zika and dengue viruses represent a major global health threat. Vaccination with the live-attenuated YF-17D strain leads to life-long protective humoral and cellular immunity. A hallmark of this vaccine is the infection of antigen-presenting cell (APC) subpopulations and their activation by multiple virus sensors. We investigated the composition and activation of APC subpopulations before and 1, 3, 7, and 14 days after vaccination with YF-17D and observed changes in the monocyte and dendritic cell (DC) subtype composition which were most pronounced on day 7 after vaccination correlating with peak expression of ISGs. To investigate which APC types in the blood are directly infected, PBMCs were infected with YF-17D in vitro and viral RNA content was measured in different APC subpopulations by RNA flow cytometry. YF-17D (+) strand RNA was detectable in DCs and monocytes and the percentage of infected cells increased with inhibition of type I IFN induction pathways. YF-17D infection induced upregulation of costimulatory molecules and MHC class II in infected and uninfected bystander cells, which was partially dependent on type I IFN induction. These results suggest that monocytes and DCs play a major role in the immune response to YF-17D infection. We propose that the success of the YF-17D vaccine is based on controlled viral replication within monocytes and DCs leading to activation and optimal presentation of viral antigens.

P.D3.04.20

Shingles vaccine works yet why are uptake rates declining in the UK?

C. M. Heffernan*, NHS England (London) Immunisation Commissioning Team;
Public Health England (London), London, United Kingdom.*

Background: In 2013, a herpes zoster (Shingles) vaccination programme was introduced in England for adults aged 70 years with a phased catch-up programme for those aged 71-79. Within 3 years, the programme had a population impact equivalent to 17000 fewer episodes of herpes zoster and 3300 fewer episodes of postherpetic neuralgia among 5.5 million eligible individuals. However, uptake rates are declining each year, particularly in London from 51.3% amongst 70 year olds in 2013/14 to 41.3% in 2016/17. NHS England (London) immunisation commissioning team have to embed the scientific evidence to improve coverage. This study reveals the barriers and facilitators to uptake of an adult vaccine that is given for individual protection rather than for herd immunity. Methods: Audit of summer 2017 and 2018 London Shingles Vaccine campaigns with London's 1301 general practices including mystery shop of 20 practices, evaluation of immunological outreach at #Shingles70 events and in-depth follow up interviews with service providers and users. Results: All 1301 received and used the campaign materials but this was not accompanied by patient invite/reminder systems. Declining vaccinating workforce and shortage of appointments were issues. Thematic analysis of interviews revealed that there was confusion around eligibility, clinicians believed that there were too many contra-indications and complexities to offer Shingles vaccine and the vaccine wasn't a priority. Patients wanted someone to talk to them about how the vaccine works. Conclusions: Improving uptake of shingles vaccine is multifaceted including targeting quality of service provision and public acceptability. Immunological outreach has an important role to play addressing vaccine hesitancy amongst healthcare professionals and patients.

P.D3.04.21

Immunogenicity of a vaccine against *Salmonella enterica* serovar Typhimurium based on Generalized Modules for Membrane Antigens (GMMA)

F. Fiorino[1], *O. Koeberling*[2], *E. Pettini*[1], *A. Ciabattini*[1], *F. Schiavo*[2], *F. Mancini*[2], *F. Necchi*[2], *L. B. Martin*[2], *D. Medaglini*[1];
[1]*University of Siena, Siena, Italy,* [2]*GSK Vaccines Institute for Global Health S.r.l., Siena, Italy.*

INTRODUCTION: Salmonella enterica serovar Typhimurium (STm) is one of the predominant causes of invasive nontyphoidal *Salmonella* (iNTS) disease. The O-antigen portion of STm lipopolysaccharide (O:4,5) has been recognized as an important target for vaccination. In this work, we characterized the murine immune response to a vaccine against STm based on the GMMA (Generalized Modules for Membrane Antigens) technology as a delivery system for O:4,5.
METHODS: C57BL/6 mice were immunized with Alhydrogel formulated STmGMMA using two doses of vaccine by subcutaneous (SC) or intranasal (IN) route. Systemic and local O:4,5-specific antibodies, serum bactericidal activity and cellular immune response were characterized.
RESULTS: Following primary immunization, high levels of O:4,5-specific serum IgG were observed with both doses of STmGMMA administered by SC route and with the higher dose by IN route. Boosting after 10 weeks induced, in all groups, an increase of O:4,5-specific serum IgG and bactericidal activity against the homologous strain. The analysis of IgG subclasses showed a balanced Th1/Th2 response following SC immunization and a Th1 response after IN. At intestinal level, the higher vaccine dose elicited O:4,5-specific IgG by SC administration, and O:4,5-specific IgA by IN route. A significant production of IL-2, IFN-γ, and IL-17A by $CD4^+$ T cells was observed in splenocytes of mice immunized with STmGMMA using both doses and administration routes.
DISCUSSION AND CONCLUSIONS: These data demonstrate the ability of the STmGMMA vaccine to induce local and systemic, humoral and cellular, immune responses and highlight the modulation of the immune response driven by different routes of immunization.

P.D4.01 Exploiting host pathogen interaction - Part 1

P.D4.01.01

Host cells respond to encephalomyocarditis virus infection by releasing diverse populations of extracellular vesicles

S. van der Grein[1], ***K. Defourny***[1], *C. Galiveti*[1], *G. Arkesteijn*[2], *H. Rabouw*[2], *F. van Kuppeveld*[2], *E. Nolte-'t Hoen*[1];
[1]*Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands,* [2]*Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, Netherlands.*

The role of extracellular vesicles (EV) in modulating the immune response to viral infection is increasingly recognized. Recently, it was demonstrated that naked viruses can be released from infected cells enclosed in EV, thereby changing their interaction with the immune system. Remarkably, both pro- and anti-viral functions have been ascribed to infected-cell derived EV. It is unknown whether this functional diversification can be attributed to different EV subpopulations released by infected cells. Therefore, we analyzed heterogeneity in the phenotype, function, and release dynamics of EV populations produced by cells infected with encephalomyocarditis virus (EMCV).
EV released by EMCV-infected cells were separated into subpopulations by differential ultracentrifugation and density gradient purification. High-resolution flow cytometry was applied to functionally separate and quantify EV subsets. In addition, protein content and infectivity of EV were analyzed by western blotting and end-point titration.
We show that EMCV infection induced the release of several EV subpopulations with a distinct protein composition indicative of separate biogenesis routes. Multiple subsets of EV transmitted infectious virus to recipient cells. Yet infection also triggered release of EV that did not efficiently convey infection. Each of these EV subpopulations were produced at different time points after infection, suggesting their tightly regulated release. Taken together, our data demonstrates the active induction of EV of mixed origin and content upon EMCV-infection. Moreover, we obtained evidence for functional diversity in virus-induced EV, by showing that EV subpopulations differed in their efficiency to transmit infection.
Supported by ERC Starting Grant 337581 and NWO grant ALWOP.351.

P.D4.01.02

Preventing type I IFN production by the aminopeptidase IRAP in neonatal alveolar macrophages in response to RSV

d. descamps[1], *C. Drajac*[1], *D. Laubreton*[1], *L. Saveanu*[2], *I. Schwartz*[1], *S. Riffault*[1];
[1]*INRA, Jouy-en-Josas, France,* [2]*INSERM, Paris, France.*

Introduction: Respiratory Syncytial Virus (RSV) is the major cause of neonatal lower respiratory tract infection. Neonatal mice have an important defect in the pulmonary production of type I interferons (IFN-I) during RSV infection compared to adults. Recently, it has been showed in adult mice that alveolar macrophages (AM) constitute the main source of IFN-I upon RSV infection. The ability of neonatal AM to produce IFN-I in response to RSV infection remains to be determined. IFN-I responses can be triggered following RSV recognition by innate immune receptors of the cell, whose their activation and intracellular trafficking are tightly controlled. Thus, Insulin-Responsive Aminopeptidase (IRAP), an protein necessary for anchoring the endosomes to the actin network, has been described to participate in regulating of IFN-I production in dendritic cells. Thus, we characterized the ability of neonatal AMs to mobilize IFN-I pathways upon RSV infection, and we determined the contribution of IRAP in this response.
Methods: Neonatal or adult AMs from IRAP-deficient ($IRAP^{KO}$) mice and wild-type (WT) were isolated and exposed ex vivo to RSV or different ligands of innate receptors to in order to compare IFN-I responses.
Results: RSV infection of adult WT AMs induced the production of IFN-I and the up-regulation of interferon-stimulated gene transcripts, while these responses are very low in neonatal AMs. However, IFN-I responses were significantly increased in neonatal $IRAP^{KO}$ AMs following RSV infection.
Conclusion: These data suggest that IRAP plays a key role in regulating responsiveness of AMs to produce IFN-I following RSV infection during the neonatal period.

P.D4.01.03

Evasion of NK cell responses by a cytomegalovirus-encoded soluble CD48 homolog

P. Engel, *P. Martínez-Vicente, D. Farré, A. Angulo;*
University of Barcelona, Barcelona, Spain.

Cytomegaloviruses (CMVs) have developed a wide range of mechanisms to subvert host immunity and establish successful long-term infections. To accomplish it, they encode a large repertoire of immune modulator genes, some of which derive from their host genomes after being captured at different points during host-virus co-evolution. CD48 is a GPI-anchored protein that contains an ectodomain composed by 2 immunoglobulin (Ig) domains. Via its N-terminal Ig domain, CD48 recognizes the cell surface receptor 2B4. Engagement of 2B4 by CD48 results in the regulation of cytotoxic T lymphocyte and NK cell functions. We have recently reported the presence of a number of CD48 homologs (vCD48) encoded by different CMVs. Here, we have characterized the three vCD48 of owl monkey CMV, showing that they are highly glycosylated transmembrane proteins which display very distinctive structural and biochemical properties. Among them, only A43, the viral CD48 that exhibits the highest amino acid identity with host CD48 is able to bind 2B4, with the two other vCD48s having diverged to perform 2B4-independent functions. Interestingly, A43 is a soluble protein, released from the cell after being proteolitically processed through its stalk region. Kinetic studies revealed that A43:2B4 interactions are of exceptional affinity and highly stable, resulting in a Koff drastically reduced as compared with that established between CD48 and 2B4. We demonstrate that purified soluble A43 is capable to efficiently abrogate CD48:2B4 interactions. Furthermore, this viral protein severely impairs 2B4 mediated-NK cell cytotoxicity. Thus, A43 acts as a functional virally-encoded CD48 decoy receptor.

P.D4.01.04

TNFAIP3 negatively regulates Cutibacterium acnes-induced inflammatory events in human epidermal keratinocytes

L. Erdei[1], *B. S. Bolla*[1], *G. Tax*[1], *E. Urbán*[2], *L. Kemény*[1,3], *K. Szabó*[3];
[1]*Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary,* [2]*Institute of Clinical Microbiology, University of Szeged, Hungary, Szeged, Hungary,* [3]*MTA-SZTE Dermatological Research Group, Szeged, Hungary, Szeged, Hungary.*

Human epidermal keratinocytes can recognize the skin microbiome, such as *Cutibacterium acnes* (*C. acnes*), through TLRs, and induce innate immune and inflammatory events. Little is known about endogenous regulators which can control these events and protect the host from the prolonged inflammation. For that, we aimed to analyze whether TNFAIP3, a negative regulator of NF-κB signaling has a role in TLR ligand and *C. acnes*-induced innate immune and inflammatory events. In our studies we used a human immortalized keratinocyte cell line (HPV-KER). We analyzed TNFAIP3 expression in response to different TLR ligands and *C. acnes*, and followed NF-κB activation using a luciferase reporter assay and by monitoring the expression changes of pro-inflammatory mediators upon TNFAIP3-silencing by real time RT-PCR, western blotting and ELISA analysis. Our results show that TNFAIP3 mRNA and protein expression significantly increased in response to different TLR ligands. By analyzing the *C. acnes*-induced signaling events in details, we found that bacterial treatment also induced a significant, transient and dose-dependent upregulation of TNFAIP3 expression, which were dependent on JNK and NF-κB pathways. Downregulation of TNFAIP3 levels by siRNA-mediated silencing increased the basal NF-κB promoter activities and the mRNA expression of selected pro-inflammatory cytokines and chemokines, such as TNFα, IL-1α, IL-6, CXCL8 and CCL5. Parallel to that, secreted IL-6, CXCL8 and CCL5 levels also increased. Based on our results, TNFAIP3 is one of the negative regulators in keratinocytes, which may control *P. acnes*-induced signaling events and play a role in the maintenance of epidermal homeostasis.

P.D4.01.05

CD8 T CELLS IN EXPERIMENTAL ZIKA VIRUS INFECTION

N. Ghabdan Zanluqui, *C. Manganeli Polonio, L. Gomes de Oliveira, C. Longo de Freitas, J. Schatzmman Peron;*
Biomedical Sciences Institute, São Paulo, Brazil.

Introduction. The relevance of Zika virus (ZIKV) infection study was highlighted by the large number of infants born with microcephaly and some adults exhibited cases of Guillain-Barré syndrome caused by ZIKV infection. It is known that ZIKV, like other flaviviruses, has the ability to modulate innate and adaptive immune response of the host. Thus, in this work we aim to evaluate the role of CD8 cells in controlling viral replication and disease progression in murine infection by ZIKV. Methods. ZIKV (BeH815744 strain) was used to infect C57BL/6 (WT) and CD8-/- mice (106 PFU). All analysis were performed at 1, 3 and 5 days post infection by PCR, flow cytometry and CBA. Results. Although high infection both WT and CD8-/- mice did not show any morbidity or clinical sign in the course of infection. Interestingly, in the peak of infection CD8-/- showed high viremia compared to WT while the opposite was observed in the spleen.
Analysis of spleen showed downregulation of type I Ifn expression and their signaling in CD8-/- mice. On the other hand, it was possible to observe in WT spleen mice an increase of CD4+ and CD8+ cells frequency, accompanied by CD8+Foxp3+ and CD4+Foxp3+ cells, while a decrease of IL-10 secretion in CD8-/- mice was detected. Conclusion. CD8 T cells could regulate type I Ifn expression which is one of the most important cytokines in controlling viral replication and T suppressors cells could be evolved in suppressing immune response against zika virus infection.

P.D4.01.06

EBV-specific CD8+ T cells are exhausted and senescent in Multiple Sclerosis patients

G. Guerrera[1], *D. F. Angelini*[1], *S. Ruggieri*[2], *F. Gargano*[1], *C. Gasperini*[2], *R. Placido*[1], *G. Borsellino*[1], *L. Battistini*[1];
[1]*Neuroimmunology Unit, Santa Lucia Foundation, Rome, Italy,* [2]*Department of Neuroscience "Lancisi", San Camillo Hospital, Rome, Italy.*

Multiple sclerosis (MS), the most common chronic inflammatory disease of the central nervous system (CNS), is associated with an increased Epstein-Barr virus (EBV) sero-prevalence and high immune reactivity to EBV. While EBV infection alone cannot explain MS development, our hypothesis is that, in susceptible individuals, defects in the control of EBV facilitate the establishment of viral infection and of continuous cycles of inflammation in the CNS, due to the recruitment and activation of inflammatory cells in the brain.
To study the immune response to EBV, we characterized the CD8+ T cells response specific for EBV lytic and latent antigens using pentamers. We measured their frequency, activation and functional state in MS patients compared with healthy donors (HD).
In MS patients, CD8+ cells specific for EBV antigens show the phenotype of terminally differentiated and senescent cells compared to HD. When stimulated with the EBV peptides corresponding to the pentamers, CD8 lymphocytes display a functional and specific response in terms of release of interferon, granzyme and CD107a. By analyzing the phenotype of these CD8+ interferon+ cells we confirm that the CD8 cells of MS patients express high levels of PD-1, compared to HD.
Thus, in MS patients, EBV-specific cells progress through terminally differentiated and functionally impaired and senescent cells, likely due to chronic viral stimulation; secondly, we find that some EBV-specific T cells are exhausted and express PD-1.

P.D4.01.07

γδ T-cells in murine Cytomegalovirus infection

A. M. Hahn[1], *S. Sell*[1], *A. Schneider*[1], *S. Brey*[1], *A. Donaubauer*[1], *M. Mach*[2], *T. H. Winkler*[1];
[1]*Nikolaus-Fiebiger Center for Molecular Medicine, Institute of Genetics, Department of Biology, University Erlangen-Nuremberg (FAU), Erlangen, Germany,* [2]*Institute for Clinical and Molecular Virology, University Hospital Erlangen, Erlangen, Germany.*

Introduction: Upon Cytomegalovirus (CMV) infection immunocompromised patients are at substantial risk for developing severe organ disease eventually leading to multi-organ failure. Existing virus escape mechanisms to anti-viral drugs plus missing approved vaccination currently designate CMV as major health issue. Previous findings showed that γδ T cells can effectively control murine CMV (mCMV), remarkably when conventional immune mechanisms (like αβT, B or Natural Killer cells) are insufficient or absent, corresponding to the immune-suppressed state in transplant recipients and neonates. In accordance with recently published data from the human system, these studies suggest adaptive features of γδ T cells.

However, recognition mechanisms, antigen specificity and the formation of a classical memory remain enigmatic. Material & Methods: To examine, to what extent the T cell receptor (TCR) participates in sensing mCMV presence, we monitored γδ TCR repertoires in TCR$\alpha^{-/-}$ mice for clonal diversity. In different organs RNA-based immune-profiling of V(D)J rearranged complementary-determining regions (CDR) from selected receptor chains (TRGV1, TRGV4, TRDV5, TRAV15/DV6) was performed in a time kinetic manner. Taking advantage of a fluorescent reporter system for TCR activation and hybridoma technology we identified γδ T cell clones which recognize mCMV infected target cells *in vitro*. Results & Conclusions: Alterations in clonality and focusing of the CDR3 length distribution after virus exposure implicate an antigen-driven response with TCR involvement. Ongoing experiments will have to define the TCR engagement in more detail via mutational studies and the exploitation of virus deletion mutants. CMV-reactive γδ T cells are a promising target for future cellular therapies.

P.D4.01.08

Association of autoantibody to rods, rings with hepatitis C virus load

Y. Lakhoua Gorgi[1], *J. Abdellatif*[1], *M. Jellouli*[1], *M. Majdoubi*[1], *L. Mouelhi*[2], *T. Ben Abdallah*[1], *I. Sfar*[1], *T. Dhaouadi*[1];
[1]*Research Laboratory in Immunology of Renal Transplantation and Immunopathology (LR03SP01), Tunis, Tunisia,* [2]*Department of Gastroenterology Charles Nicolle hospital, Tunis, Tunisia.*

INTRODUCTION: Chronic infection with hepatitis C virus (HCV) is an indication for treatment with Ribavirin, a nucleoside analogue of guanosine. The cellular targets of Ribavirin are 2 enzymes (CTPS1 and IMPDH2) which are essential for the CTP and GTP nucleosides' synthesis. It has been reported that in treated patients, the appearance of rare autoantibodies directed against these 2 enzymes and giving the appearance of Rods and Rings (RnR) is generally correlated with a viral escape to treatment with Ribavirin. **MATERIAL AND METHODS**: In this context, anti-RnR antibodies (Ab) were detected by IFI on HEp-2 cells in 142 HCV patients under Ribavirin: 74 patients with positive HCV-PCR(G1) and 68HCV-PCR negative patients (G2) matched in age and sex with G1 and served as controls. **RESULTS**: The frequency of anti-RnR Ab was significantly higher in HCV-PCR positive patients (25.7%) than in those with negative HCV-PCR (0%), p<10E-5. Furthermore, in G1, the viral load was significantly higher in patients with anti-RnR Ab (6105378.32 IU/ml vs 380527.38 IU/ml), p=3.13 10E-6. Nevertheless, the presence of anti-RnR Ab was not correlated neither to gender nor to age. **CONCLUSION**: Anti-RnR Ab seems to be associated with increased replication of HCV and would be predictive of viral escape under Ribavirin.

P.D4.01.09

Cyokine responses upon Bovine Respiratory Syncytial Virusand Bovine Viral Diarrhea Virus vaccination in dairy cows

S. Lee*, Y. Kim, S. Ryu, A. Prasad;*
Chonbuk National University, Iksan, Korea, Republic of.

Bovine respiratory disease (BRD) is defined as a "disease complex", which is usually caused by a variety of pathogens including Bovine Respiratory Syncytial Virus (BRSV), ParaInfluenza 3 (PI3), and Bovine Viral Diarrhea Virus (BVDV). The key to reduce BRD is to vaccinate against these viruses. Although vaccination is an effective measure in reducing the risk of BRD in cattle, BRD losses remain significant. Increasing the efficacy of vaccination depends on elucidating the protective immune response to different antigens included in vaccines and understanding the cytokine responses which reflect complicated immune responses against vaccinated antigens. This study evaluated the serum antibodies present in dairy cows following vaccination with BRSV, BVDV and PI3. Antibody titers and cytokines were measured in more than 100 cows at 0, 1, 4, 24 weeks post vaccination. Upon vaccination, the levels of antibodies against viral respiratory pathogens were significantly enhanced in serum and innate cytokines such as TNF-α and IL-6 were also increased at 1 wk. Cytokines related with helper T cells including IFN-γ, IL-4 and IL-17 were also modulated by vaccination and correlated with antibody levels in serum. The present study may provide better understanding of immune responses against viral pathogens related with BRD upon vaccination.

P.D4.01.10

Mast cells promote thrombocytopenia during dengue virus infection through the release of serotonin.

M. Masri*, C. Mantri, A. Rathore, A. St. John;*
Duke-NUS Medical School, Singapore, Singapore.

Introduction: Thrombocytopenia, a reduction in platelet counts, is a classical trait of dengue fever (DF), which is caused by infection by dengue virus (DENV). DENV can activate mast cells (MCs), which are innate immune sentinels. This results in the release of MC mediators that can influence the severity of dengue vascular leakage. We hypothesized that MC-derived products also contribute to thrombocytopenia during DENV infection.
Materials and methods: Wild type (WT) and MC-deficient (Sash) mice were infected with DENV. Blood and spleens were harvested post-infection for analysis of platelet activation and turnover by flow cytometry. WT mice were treated with MC stabilizer, ketotifen, or $5HT_{2A}$ antagonist, ketanserin. Sash mice were reconstituted with MCs or administered exogenous serotonin to determine the role of mast cell derived serotonin during DENV infection.
Results: DENV infection of WT mice induced thrombocytopenia, which was absent in Sash mice and reduced in mice treated with ketotifen. Reconstitution of the Sash mice with MCs restored the phenotype of thrombocytopenia. Pharmacological inhibition of serotonin with the $5HT_{2A}$ receptor antagonist, ketanserin, in DENV-infected WT mice reduced thrombocytopenia compared to vehicle treated mice. Conversely, treatment of DENV-infected Sash mice with exogenous serotonin restored the thrombocytopenic phenotype that is absent in Sash mice.
Conclusions: Our findings have demonstrated that MC release of serotonin contributes to thrombocytopenia during DENV infection, revealing a potential therapeutic target of disease.

P.D4.01.11

A new in vivo model to study protective immunity to Zika virus infection in mice with intact type I interferon signaling

L. Nazerai*, A. Skak Schøller, P. Overbeck Sharma Rasmussen, S. Buus, A. Stryhn, J. Pravsgaard Christensen, A. Randrup Thomsen;*
Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark.

The association between recent Zika virus (ZIKV) infection and severe neurological complications, microcephaly in the fetus and Guillain-Barré syndrome in adults, underscores the necessity for a protective vaccine. Unlike the situation in humans, ZIKV can only replicate effectively in mice when type I IFN signaling is interrupted. As type I IFN also impacts the adaptive immune response, mice with such a defect are not optimal for a comprehensive immunological analysis.
In this report, we show that even in wild-type (WT) mice, (intracranial) i.c. infection with low doses of ZIKV causes marked local virus replication and lethal encephalitis in naïve mice. Furthermore, peripheral infection of WT mice with low doses of virus induces a significant immune response, which provides long-lasting protection of WT mice from a fatal outcome of subsequent i.c. challenge. Therefore, combining peripheral priming with later i.c. challenge represents a new approach for studying the adaptive immune response to ZIKV in mice with intact type I IFN response. Using a combination of adoptive transfer, antibody-based cell depletion, and gene targeting, we show that the key protective factor in type I IFN replete mice is humoral immunity. CD8 T cells are not essential in mice with preformed specific antibodies, but under conditions where initial antibody levels are low, effector CD8 T cells may play a role as a back-up system. These results have important implications for our understanding of natural immunity to ZIKV infection and for Zika vaccine design.
Grant: Lundbeckfonden
Fellowship: Department of Immunology and Microbiology (ISIM)-University of Copenhagen

P.D4.01.12

Reactivity of the immune system in rabbits experimentally infected with antigenic variants of RHDV (rabbit haemorrhagic disease virus).

P. Niedzwiedzka-Rystwej[1], *B. Tokarz-Deptuła*[1], *W. Deptuła*[2];
[1]*University of Szczecin, Faculty of Biology, Department of Immunology, Szczecin, Poland,* [2]*University of Szczecin, Faculty of Biology, Department of Microbiology, Szczecin, Poland.*

The aim of the study was to evaluate the immunogenicity of 6 selected RHDVa (Vt97, Triptis, Hartmannsdorf, Pv97, 9905 and 72V/2003) obtained in 1996-2003, based on selected natural and acquired immunity parameters. Clinical symptoms and mortality were also recorded. The experiment was performed on 120 rabbits of Polish mixed breed rabbits. Blood and serum was collected at hour 0, followed by 4, 8, 12, 24, 36, 48, 52, 56 and 60 h p.i. Analyzing the picture of changes in natural immunity parameters, the RHDVa strains differ from each other enough to be divided into three groups: with the highest amount of changes - Vt97 and Pv97, the average number of changes - Triptis and 72V/2003 and a small amount of changes - Hartmannsdorf and 9905. In contrast, in case of acquired immunity, no differences were found. No clinical symptoms were recorded, as the infection was rapid and severe, causing mortality in 24 h p.i. (Hartmannsdorf, 72V/2003) and in 36 h p.i. (Vt97, Triptis, Pv97, 9905) at 90 -100%. The picture caused by RHDVa is different both between the analyzed RHDVa (mainly in natural immunity), but also from the image of previously tested classical RHDV. The reasons for these differences may lie in the dissimilarity of RHDVa from RHDV also in genetic terms, as nucleotide changes within the VP60 protein between RHDV and RHDVa have been confirmed. However, the time of receiving RHDVa strains does not confirm the observations with classical RHDV strains, where time of isolation significantly influenced their diversity.

P.D4.01.13

Upregulation of intestinal Tumor Growth Factor-beta1 is associated with increased intestinal epithelial cell apoptosis in pathogenic simian immunodeficiency virus infection

B. Pahar[1], *D. Pan*[1], *S. K. Srivastav*[2], *W. Lala*[1], *A. Das*[1];
[1]*Tulane National Primate Research Center, Covington, United States,* [2]*Tulane University, New Orleans, United States.*

Mucosal Transforming Growth Factor-β1 (TGF-β1), a pleiotropic, potent immunoregulatory cytokine, demonstrated to manage phosphorylated AKT and IFNγ expressions, are associated with intestinal epithelial cells (iECs) survival in macaque colon explants and suggest a potential role of mucosal TGF-β1 in regulating intestinal homeostasis and iEC integrity. It is important to monitor and further explore the role of mucosal TGF-β1 in HIV/SIV pathogenesis and HIV/SIV enteropathy, which may lead to the development of improved therapeutic strategies to prevent iEC damage and systemic immune activation during acute and chronic infection. Our data showed an increased production of intestinal TGF-β1 in T-, B- and non-T/B cell populations during acute and chronic SIV infection in rhesus macaques, without a change in the expression of TGF-βRII. The increased levels of immunosuppressing TGF-β1 were also associated with increased production of IFNγ, suggest the lack of TGF-β1 mediated anti-inflammatory responses in SIV infection. An appropriate balance between inflammatory and anti-inflammatory cytokine responses are crucial for maintenance of a successful immune responses in HIV infection. TGF-β1 induced immune defects contribute to intestinal inflammation, loss of tight junction protein, and apoptosis by overexpression of SMAD3, and downregulation of inhibitory SMAD7 transcription factors. Together, these results indicate that SMAD mediated pathway play a crucial role in regulating TGF-β1 expression and that was thought to be a key contributor to the dysfunction of CD4+ and CD8+ T-cells, iEC apoptosis and disease progression.

P.D4.01.14

Immune modulation in inflammatory bowel disease; the role of IgA-coated microbiota

L. Pedró-Cos*, A. Breedveld, H. Schuster, R. Mebius, D. Budding, M. van Egmond;*
VUmc, Amsterdam, Netherlands.

Immunoglobulin A (IgA) is the most abundant immunoglobulin in mucosal areas and fundamental in maintaining gastrointestinal (GI) tract homeostasis. IgA opsonizes pathogens thereby facilitating uptake by immune cells including neutrophils and dendritic cells (DCs) through FcαRI activation. Inflammatory bowel disease patients have a disturbed intestinal microbiota and their tissue is highly infiltrated with neutrophils. Unfortunately not much is known about the interaction of bacteria with neutrophils and different DC subsets in the GI tract. Here we evaluate the effect of IgA-coated bacteria on immune cells. In the future we aim to identify DC subsets in inflamed intestinal tissue and evaluate their relationship with neutrophils.
Specific IgA in pooled human serum against *E. coli*, *S. aureus*, *S. typhimurium*, *B. breve* and *B. longum* was measured using flow cytometry. Neutrophils, monocytes and monocyte derived (mo)DCs were stimulated with IgA-coated bacteria to measure their phagocytic capacity after 30min using flow cytometry. After 24h stimulation, supernatant was collected for cytokine production measurement using ELISA.
Serum IgA against *E. coli*, *S. aureus*, *S. typhimurium*, *B. breve* and *B. longum* was present. Neutrophil phagocytic capacity was enhanced when bacteria were IgA-coated while moDCs preferably phagocytose IgG-coated bacteria. Activation of monocytes was increased after 24h bacterial stimulation showing enhanced production of IL-6. *B. longum* induced higher IL-10 production by monocytes compared to *S. typhimurium*. Surprisingly, IgA-coated *B. longum* induced higher levels of IL-12p40 production by monocytes. These results suggest differential effector functions of (IgA-coated) bacteria on immune cells.

P.D4.01.15

Circadian rhythms control lung inflammation in influenza infection

S. Sengupta[1], *S. Y. Tang*[1], *C. B. Lopez*[2], *S. Nayak*[1], *J. Devine*[1], *G. Grant*[1], *E. Genoyer*[2], *G. A. FitzGerald*[1];
[1]*University of Pennsylvania, Philadelphia, United States,* [2]*University of Pennsylvania School of Veterinary Medicine, Philadelphia, United States.*

Influenza is a leading cause of respiratory mortality and morbidity. While inflammation is necessary for fighting infection, a fine balance of anti-viral defense and host tolerance is crucial to the successful outcome. Circadian rhythms form an anticipatory system wherein various aspects of cellular physiology and behaviors oscillate across a 24hrs period; these rhythms have been known to modulate the immune responses. However, the role of circadian rhythms in influenza infection is not well known. To elucidate this role, we infected C57bl6 littermates with influenza virus (PR8) either at the beginning of their active phase (ZT11) or at the beginning of their rest phase (ZT23). Mice infected at ZT11 had more than fourfold higher mortality, more weight loss and worse clinical scores than mice infected at ZT23. This was the result of exaggerated inflammation as evident in higher bronchoalveolar lavage cell counts (including more neutrophils on day 2 post-infection) and worse lung histology on day 6. Interestingly, NK cells were present in higher numbers in the ZT23 than in the ZT11 group at the early phase of infection. Further, pathway analyses of our transcriptomic data supported the role of higher inflammation in the ZT11 group. Thus, circadian regulatory networks, make the host at ZT11 mount an inefficient viral clearance response, wherein more inflammation is needed to achieve similar clearance of the pathogen, resulting in more host injury and death. These data suggest that the circadian regulation of host inflammation and tolerance, rather than viral burden determines the outcome of influenza infection.

P.D4.01.16

The evidence of hepatitis E virus in wild rats from Lithuania

M. Simanavicius[1], *K. Juskaite*[1], *A. Verbickaite*[1], *M. Jasiulionis*[2], *P. L. Tamosiunas*[1], *R. Petraityte-Burneikiene*[1], *A. Zvirbliene*[1], *R. G. Ulrich*[3,4], *I. Kucinskaite-Kodze*[1];
[1]*Vilnius University Life Sciences Center Institute of Biotechnology, Vilnius, Lithuania,* [2]*Nature Research Centre, Vilnius, Lithuania,* [3]*Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Novel and Emerging Infectious Diseases, Greifswald - Insel Riems, Germany,* [4]*German Center for Infection Research (DZIF), Hamburg-Luebeck-Borstel-Insel Riems, Germany.*

Rat hepatitis E virus (HEV) is an orthohepevirus which is related to other HEV found in humans and other mammals. It was first identified in Norway rats (*Rattus norvegicus)* from Germany in 2010, and then it was detected in Black rats (*Rattus rattus)* and Norway rats from USA, China, Indonesia, Vietnam and many European countries. In this study, we describe molecular and serological investigations of Black and Norway rats trapped in Lithuania for infections with rat HEV and human HEV genotypes 1-4. Rat HEV-specific real-time reverse transcription PCR (RT-qPCR) analysis of rat liver samples revealed the presence of rat HEV. In contrast, a RT-qPCR specific for HEV genotypes 1–4 did not reveal any positive samples. A nested broad spectrum RT-PCR was used for a confirmation of rat HEV infection with a subsequent sequencing of the amplified rat HEV genomic fragment. Phylogenetic analysis revealed a clustering of all newly identified rat HEV sequences with Norway rat-derived rat HEV sequences from Germany and a Black rat-derived sequence from Indonesia within the species *Orthohepevirus C*. An indirect ELISA using a yeast-expressed truncated rat HEV capsid protein variant revealed even more seropositive than RT-qPCR positive samples indicating a high rate of rat HEV circulation in the rat population examined. In conclusion, the current investigation confirms rat HEV infections in Norway and Black rats in Lithuania and the non-persistent nature of infection. This research was funded by the Lithuanian Science Council, grant number MIP-039/2015.

P.D4.01.17

Evaluation of the carriage of hepatitis B surface antigen in hospital personnel

M. D. SYLLA NIANG[1,2], *B. Mbengue*[1], *M. Mbow*[1], *M. Thiam*[1,2], *R. Derwiche*[1], *N. N. Diouf*[2], *O. Boye*[2], *T. N. Dieye*[1], *A. Dieye*[1];
[1]*Cheikh Anta Diop University of Dakar - SENEGAL, Dakar, Senegal,* [2]*General Hospital of Grand-Yoff, Dakar, Senegal.*

Introduction: Hepatitis B virus (HBV) infection is the second most common carcinogen factor after tobacco. The high concentration of HBV in biological secretions and its resistance in the external environment promote its high transmission capacity. Such characteristics make hospital staff at risk to HBV infection. The objective of this study was to evaluate the immunological status to HBV of hospital staff from a public health institution in Senegal.
Material and methods: A retrospective, descriptive and analytical study was conducted from June to August, 2015 at General Hospital of Grand-Yoff in Senegal. Were included in this study 319 out of 654 hospital staff members who attended the 2015 annual company medical check-up. Determination of HBsAg was performed using qualitative immunochromatographic test. Data were analyzed using Excel 2011 and Statview 5.0 software.
Results: The participation rate was 48.77%. The average age of the staff was 45± 2 years. The prevalence of HBsAg in the study population was 13%. HBsAg carriers were mostly paramedics (60%) and most of them (70%) have been working at the hospital for more than 10 years. Statistical analysis revealed that there was no relationship between HBV infection and the work duration at the hospital. Among the infected staff, 92% were married and the most represented age group was between 39 and 45 years.
Conclusion: The prevalence of HBsAg among hospital staff remains high. Preventive measures such as vaccination of people unprotected from HBV and awareness of the risks associated with practice in the hospital environment are needed.

P.D4.01.18

Effect of latent cytomegalovirus (CMV)-infection on the immune response to influenza with age

S. P. H. van den Berg[1,2], *R. J. Jacobi*[1], *M. Hendriks*[1], *R. van Schuijlenburg*[1], *K. Warmink*[1], *L. Beckers*[1], *N. M. Nanlohy*[1], *A. Wong*[1], *M. Knol*[1], *J. A. Borghans*[2], *J. van Beek*[1], *D. van Baarle*[1,2];
[1]*National Institute for Public Health and the Environment, Bilthoven, Netherlands,* [2]*University Medical Center Utrecht, Utrecht, Netherlands.*

Older adults are at higher risk for influenza-virus infection and for influenza-related death compared to younger adults. Unfortunately, influenza-vaccine responses are impaired in older adults due to ageing of the immune system (immunosenescence). Latent infection with cytomegalovirus (CMV) is generally thought to aggravate this state of immunosenescence. We studied whether CMV-infection impairs the immune response to influenza-virus. The effect of CMV-infection on the influenza-vaccine antibody response has been investigated before, with very contradicting results. Meta-analysis of these studies revealed a trend towards a negative effect of CMV-seropositivity on the response to influenza vaccination. Funnel-plot analysis suggests, however, that this is due to publication bias. Differences between studies might be due to confounding effects of preexisting immunity, which influences immune responses to seasonal influenza vaccines. Therefore, we investigated the influence of CMV-infection on the antibody response to the pandemic influenza vaccine of 2009, to which pre-existing immunity was negligible. Despite a negative effect of age, no effect of CMV-infection on the influenza-vaccine response was observed. Finally, we investigated the effect of CMV-infection on influenza virus-specific T-cells in a cohort of influenza virus-infected elderly. Despite a clear effect of CMV-infection on the phenotype of the total T-cell pool, CMV-infection did not impair the T-cell response to influenza-virus infection. Unexpectedly, the height of the T-cell response to CMV and influenza virus correlated positively, showing that the CMV-host balance is still not fully understood. Altogether, we find no evidence in human for impairment of the immune response to influenza vaccination or infection by CMV.

P.D4.01.19

Prevalence of broadly neutralizing antibody responses in HIV-1 infected injecting drug users

Z. Euler[1,2,3], *T. van den Kerkhof*[1,3], *R. Kouyos*[4], *D. Tully*[2], *T. Allen*[2], *R. Sanders*[1], *A. Trkola*[4], *H. Schuitemaker*[1,3], ***M. J. van Gils***[1];
[1]*Academic Medical Center, Amsterdam, Netherlands,* [2]*Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, United States,* [3]*Janssen Pharmaceuticals, Leiden, Netherlands,* [4]*Institute of Medical Virology, Zurich, Switzerland.*

Understanding the factors involved in the development of broadly neutralizing antibody (bNAb) responses in natural infection can guide vaccine design aiming to elicit protection against HIV-1 acquisition. Most of the studies to identify and study development of bNAb responses were performed in individuals who had become infected via homo- or heterosexual HIV-1 transmission, however the prevalence and characteristics of bNAb responses in injecting drug users (IDUs) remains to be established.
A retrospective cohort study on the prevalence of bNAb responses in HIV-1 infected individuals, who reported injecting drug use as the only risk factor (50 male and 35 female participants of the Amsterdam Cohort Studies) was conducted.
The study revealed a significantly lower prevalence of bNAb responses compared to MSM, which was no longer evident when women were excluded from the IDU group. Interestingly, more elite neutralizers were found in the IDUs with 6% of male IDUs being elite neutralizers as compared to only 0.3% amongst MSM and 0% of female IDUs. Gender, transmission route and CD4+ count at setpoint, were independently associated with bNAb responses but not viral load for the IDUs or HIV-1 envelope glycoprotein sequence diversity within the first year of infection. Similar observations were seen in the Swiss Cohort, indicating that injecting drug use may influence the development of potent humoral immune responses, with a stronger effect in females.
Our data reveal that the emergence of bNAbs may be dependent on multiple factors, not only host or viral but also behaviour.

P.D4.01.20

Identification of new biomarkers to distinguish between a bacterial or viral infection in children

J. Zandstra[1,2], *M. H. Jansen*[3,2], *S. Zeerleder*[1,4], *T. W. Kuijpers*[2,5];
[1]*Dept of Immunopathology, Sanquin Blood Supply, Division Research, Amsterdam, Netherlands,* [2]*Dept of Pediatric Hematology, Immunology & Infectious diseases, Emma Children's Hospital, AMC, Amsterdam, Netherlands,* [3]*Department of Experimental Immunology, Academic Medical Center, Amsterdam, Netherlands,* [4]*Department of Hematology, Academic Medical Center, Amsterdam, Netherlands,* [5]*Dept of Blood Cell Research, Sanquin Blood Supply, Division Research, Amsterdam, Netherlands.*

The biggest cause of death in children under 5 years consists of infection. The most common presenting symptom of infection is fever. Most of febrile illnesses are caused by viral infections at this age. However a small number are life-threatening bacterial infections, such as meningitis or pneumonia. In the clinic it may be difficult to distinguish between a bacterial or viral infection based on clinical grounds. This results in unnecessary treatment with antibiotics when they are suffering from a viral infection out of fear of missing a bacterial infection. There is an urgent need of the development of improved methods to distinguish between bacterial and viral infections. In a prospectively, we focused on identification of new discriminators of bacterial and viral infection. We performed a multiplex protein assay with 27 candidate markers and ELISA to validate known biomarkers. In our cohort of 150 bacterial and viral plasma samples we have confirmed by ELISA that C-reactive protein and neutrophil protein elastase is increased in bacterial infections compared to viral infections. We found several pro-inflammatory proteins elevated in plasma from children with meningococcal infection. Also, neutrophil-derived S100A12 was significantly increased in bacterial infections, compared to viral infection. Further analysis into these markers is needed to get insight in the predictive value of these parameters. Our data clearly indicate that a reliable diagnostic tool based on a multiplex protein test will lead to a more accurate diagnosis to reduce hospital admissions and avoid overtreatment with antibiotics in febrile disease in pediatrics.

P.D4.02 Exploiting host pathogen interaction - Part 2

P.D4.02.01

β-defensin as mast cell phenotype and activity modulator

J. Agier[1], *S. Rożalska*[2], *M. Wiktorska*[3], *P. Żelechowska*[1], *E. Brzezińska-Blaszczyk*[1];
[1]*Department of Experimental Immunology, Medical University of Lodz, Lodz, Poland,* [2]*Department of Industrial Microbiology and Biotechnology, University of Lodz, Lodz, Poland,* [3]*Department of Molecular Cell Mechanisms, Medical University of Lodz, Lodz, Poland.*

Background. Defensins play a crucial role as components of the early host defense against bacterial, viral, and fungal invasion but new research has cast light on alternative functionalities, including immunomodulatory activities. Given that mast cells (MCs) are firmly efficient effector cells in microbial elimination and play an essential role in orchestrating inflammatory response during infection, this study analyzes hBD-2-induced expression of RIG-I, NOD1, and NOD2 receptors and evaluates the effect of this peptide on the pro-inflammatory response in *in vivo* differentiated mature tissue MCs. Materials and methods. All experiments were carried out *in vitro* on freshly isolated peritoneal MCs obtained from female albino Wistar rats. qRT-PCR, flow cytometry, and confocal microscopy were used to evaluate both constitutive and hBD-2-induced expression of receptors. ROS was determined using H_2DCFDA, and chemotaxis assay was used to define the MC migratory response. Standard procedure assessed histamine release. Results. hBD-2 enhances the expression and induces translocation of the studied receptors and directly activates the pro-inflammatory and migratory responses of native MCs. Conclusion. These data suggest that hBD-2 might augment MC capability and sensitivity to RLR and NLR ligands and strengthen the role of MCs in inflammation. Supported by the Medical University of Lodz (grant no 503/6-164-01/503-61-001).

P.D4.02.02

Investigating metabolic mechanisms regulating collagen breakdown in tuberculosis

R. M. Asher, *J. S. Friedland;*
Imperial College London, London, United Kingdom.

Introduction: Tuberculosis (TB) is a global pandemic. Morbidity and mortality in TB result from inflammatory tissue destruction, driven by matrix metalloproteinases (MMP). Patients also experience profound weight loss. TB-infected macrophages display the Warburg effect, a metabolic shift from oxidative phosphorylation to aerobic glycolysis. However, the relationship between innate inflammation and cellular metabolism in TB remains poorly defined.
Methods: Primary normal human bronchial epithelial cells (NHBE) or monocyte-derived macrophages (MDM) were incubated with specific metabolic inhibitors, or transfected with siRNA. Cells were then directly infected with live, virulent TB or stimulated with conditioned media from TB-infected monocytes (CoMTB). Protein secretion, gene expression and functional tissue damage were measured by ELISA, luminex, zymography, real-time PCR and DQ collagen assay.
Results: The glycolysis inhibitor, 2-deoxyglucose (2DG), reduced gene expression and secretion of the key collagenase, MMP-1, in a dose-dependent manner. There was a 7-fold drop in MMP-1 secretion in CoMTB-stimulated NHBE ($p<0.0001$) and a 5-fold drop in TB-infected MDM ($p<0.0001$). This was accompanied by a functional decrease in collagen breakdown. 2DG also decreased IL-1β ($p=0.0001$) and IL-10 ($p<0.0001$) and increased TNF-α ($p=0.0012$) in TB-infected MDM. Enhanced transcription factor HIF-1α expression in CoMTB-stimulated MDM was attenuated by 2DG. MMP-1 and IL-1β secretion were decreased by inhibiting the metabolic regulator AMPK and increased by Pi3-kinase pathway blockade.
Conclusion: Our data show that glycolysis is a key modulator of MMP-1, cytokines and HIF-1α in TB. AMPK and Pi3-kinase also have central regulatory roles.
Research was funded by Medical Research Council (UK) and Mason Medical Research Foundation.

P.D4.02.03

Plasma cytokines CXCL9, CXCL10 and IL1RA correlate with disease severity in a controlled typhoid human challenge model

A. J. Barton[1], *M. Gibani*[1], *E. Jones*[1], *S. Camara*[1], *Y. Rosenberg-Hasson*[2], *I. Mohorianu*[1], *G. Obermoser*[3], *J. Galan*[4], *A. J. Pollard*[1];
[1]*University of Oxford, Oxford, United Kingdom,* [2]*Human Immune Monitoring Center, Stanford University, Stanford, United Kingdom,* [3]*Human Immune Monitoring Center, Stanford University, Stanford, United States,* [4]*Yale University, New Haven, United States.*

Introduction: *Salmonella enterica* serovar Typhi (*S*. Typhi) is a human-restricted pathogen estimated to cause 20 million cases of typhoid fever each year. The contribution of the typhoid toxin, a virulence factor specific to typhoidal *Salmonella*, to host-pathogen interactions *in vivo* is not well characterised. Here we investigate the changes in cytokine profile induced by wild-type and toxin-deficient *S*. Typhi challenge, and their correlation with disease severity.
Methods: 40 healthy adult volunteers were randomised to receive oral challenge wild-type Quailes strain *S*. Typhi or an isogenic typhoid toxin-deficient mutant with an attack rate of 75% (30/40). Post-challenge changes in plasma cytokine profile over a two week time series were assayed using a 62-plex Luminex system. Raw intensities were normalised, and log fold changes relative to baseline were compared using linear modelling.
Results: 37% of cytokines were differentially regulated in acute disease. At time of diagnosis, IL-12 was significantly upregulated in the toxin-negative group but not in the wild-type group. CXCL10 and IL1RA were positively correlated with temperature (R = 0.9 and R = 0.8) and CXCL9, CXCL10 and IL1RA with aggregate self-reported symptom scores.
Conclusions: Based on this extensive cytokine study, absence of the toxin may affect Th1 differentiation through IL-12. The correlation between disease severity and CXCR3 ligands CXCL9 and CXCL10 could relate to their action on T cell migration, while endogenous antipyretic IL1RA may have been upregulated to counteract the inflammatory action of IL-1.

P.D4.02.04

Anti-Cytomegalovirus IgG Antibody Titer is Positively Associated with Advanced T Cell Differentiation and Coronary Artery Disease in End-Stage Renal Disease

Y. Chiu[1,2], *K. Shu*[1], *I. Chen*[1], *F. Lay*[1];
[1]*Far Eastern Memorial Hospital, New Taipei City, Taiwan,* [2]*National Taiwan University, Taipei, Taiwan.*

Background: Accumulating evidence indicates that persistent human cytomegalovirus (HCMV) infection is associated with several health-related adverse outcomes including atherosclerosis and premature mortality in individuals with normal renal function. Patients with end-stage renal disease (ESRD) exhibit impaired immune function and thus may face higher risk of HCMV-related adverse outcomes. Whether the level of anti-HCMV immune response may be associated with the prognosis of hemodialysis patients is unknown. Results: Among 412 of the immunity in ESRD study (iESRD study) participants, 408 were HCMV seropositive and were analyzed. Compared to 57 healthy individuals, ESRD patients had higher levels of anti-HCMV IgG. In a multivariate-adjusted logistic regression model, the log level of anti-HCMV IgG was independently associated with prevalent coronary artery disease (OR=1.93, 95% CI=1.2~3.2, p=0.01) after adjusting for age, sex, hemoglobin, diabetes, calcium phosphate product and high sensitivity C-reactive protein. Levels of anti-HCMV IgG also positively correlated with both the percentage and absolute number of terminally differentiated CD8+ and CD4+CD45RA+CCR7- TEMRA cells, indicating that immunosenescence may participate in the development of coronary artery disease. Conclusion: This is the first study showing that the magnitude of anti-HCMV humoral immune response positively correlates with T cell immunosenescence and coronary artery disease in ESRD patients. The impact of persistent HCMV infection should be further investigated in this special patient population.

P.D4.02.05

The potential role of Th17-like immune responses in Johne's disease positive cows

J. L. DeKuiper*, P. M. Coussens;*
Michigan State University, East Lansing, United States.

Johne's disease (JD) is a chronic gastrointestinal disorder of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Later stages of JD coincide with a classical Th2-like immune response. Defining importance of a classical Th1-like response in JD has been more difficult. A possibility exists that non-classical responses, such as a Th17-like response, might be important in MAP immunity. Indeed, mRNAs encoding the cytokines IL-23 and IL-17a are significantly elevated in PBMCs from MAP test positive (JD+), MAP test negative (JD-) cows, and subclinical JD+ CD4+ T-cells (IL-17a only), after MAP antigen stimulation. IL-23 and IL-17a production have been associated with Th17-like responses. Th17 cells are also defined by expression of IL-23 receptor (IL-23R). Surface staining for T-cell type (CD4, CD8, TCR1 (Yδ T-cell)) and IL-23R was performed and analyzed via flow cytometry to determine the relative prevalence of potential Th17 cells in PBMCs from JD+/- cows. Fresh PBMCs from JD+ cows (*n=12*) contained a significantly higher proportion of IL23R+ cells than PBMCs from JD- (*n=12*) (*p<0.05*). However, ELISA results for IL-17a revealed higher concentrations of IL-17a secreted from PBMCs stimulated with MAP (*n=20*) than from PBMCs not stimulated with MAP (*n=20*), regardless of JD status (*p<0.0001*). Plasma from highly infected JD+ (*n=20*) cows revealed significantly less IL-17a circulating in the periphery than in JD- cows from two distinct sources (*n=18 and n=20*) (*p>0.05 and <0.013, respectively*). This data suggests that Th17 cells may indeed play a role in early immune responses to MAP infection and development or control of JD.

P.D4.02.06

Clustering of CD8 T cells around malaria-infected hepatocytes is rapid and is driven by antigen-specific T cells

V. V. Ganusov[1], *R. Kelemen*[2], *H. Rajakaruna*[1], *I. Cockburn*[3];
[1]*University of Tennessee, Knoxville, United States,* [2]*nstitute of Science and Technology, Vienna, Austria,* [3]*Australian National University, Canberra, Australia.*

Malaria begins when Plasmodium-infected mosquitoes inject malaria sporozoites while searching for blood. Sporozoites migrate from the skin via blood to the liver, infect hepatocytes, and form liver stages. In mice, vaccine-induced activated or memory CD8 T cells are capable of locating and eliminating all liver stages in 48 hours, thus preventing the blood-stage disease. However, rules of how CD8 T cells are able to locate all liver stages in a limited timeframe remains poorly understood. We recently reported formation of clusters consisting of variable numbers of activated CD8 T cells around Plasmodium yoelii (Py)-infected hepatocytes. Using a combination of experimental data and mathematical models we now provide additional insights into mechanisms of formation of these clusters. We show that a model in which cluster formation is driven exclusively by T-cell-extrinsic factors, such as variability in ``attractiveness'' of different Py-infected cells, cannot explain distribution of cluster sizes in different experimental conditions. In contrast, the model in which cluster formation is driven by the positive feedback loop (i.e., larger clusters attract more T cells) can accurately explain the available data. Mathematical modeling also suggested that formation of clusters occurs rapidly, within few hours after adoptive transfer of T cells, thus illustrating high efficiency of T cells in locating their targets in complex peripheral organs such as the liver. Taken together, our analysis provides novel information into the mechanisms driving the formation of clusters of antigen-specific CD8 T cells in the liver.

P.D4.02.07

Innate recognition of heat-stable ligands from *Orientia tsutsugamushi* by C-type lectins

V. Hefter[1,2], *Z. Orfanos*[1], *S. Mayer*[3], *B. Lepenies*[3], *C. Keller*[1,2];
[1]*Institute of Virology, Philipps University Marburg, Marburg, Germany,* [2]*German Centre for Infection Research at the Institute of Virology, Philipps University Marburg, Marburg, Germany,* [3]*Research Centre for Emerging Infections and Zoonosis, University of Veterinary Medicine, Hannover, Germany.*

Orientia tsutsugamushi, an obligate intracellular Gram-negative bacterium causing the neglected febrile disease scrub typhus, elicits chemokine and cytokine production by heat-stable ligands via NF-κB-dependent pathways in phagocytes. It has not been studied how recognition by receptors other than Toll-like (TLR) and NOD-like receptors shape the inflammatory response to *Orientia*. *Orientia* has an atypical cell wall composition with high amounts of neutral saccharides in its outer membrane, which could predispose for recognition by C-type lectin receptors (CLR).
In order to screen for potential CLR ligands, we used purified heat-inactivated *Orientia* in a FACS-based interaction assay involving a library of 12 mouse and 4 human CLRs. We identified four mouse candidate receptors binding to *Orientia*. Binding to mouse Mincle was EDTA-sensitive and thus shown to be specific. Mincle was therefore chosen for further investigations.
Bone marrow-derived dendritic cells (BMDC) from C57BL/6 mice stimulated with inactivated *Orientia* showed an increasing, dose-dependent induction of *mincle* mRNA over 24 hours. Furthermore, upon stimulation significantly higher levels of TNF-α were seen in Mincle-deficient BMDC compared to the C57BL/6 wildtype, suggesting an inhibitory effect of Mincle on NF-κB-mediated cytokine production.
Combined, these results point toward an initial upregulation of Mincle by another receptor, e.g. a TLR, before the former can mediate its inhibitory effect. We aim to provide further insight into the role of Mincle in the recognition of *Orientia tsutsugamushi*.

P.D4.02.08

The role of ISG15 in proteasomal degradation and MHC class I antigen presentation

***T. Held**, M. Groettrup;*
Division of Immunology, Konstanz, Germany.

Interferon stimulated gene 15 (ISG15) is an interferon (IFN)-α/β-induced ubiquitin-like protein. It exists as an intracellular and extracellular molecule, as well as conjugated to target proteins (ISGylation). Both free and conjugated ISG15 exhibit antiviral activities against a wide range of viruses. Evidence indicates that ISGylation mostly targets newly synthesized proteins, as its E3 ligase Herc5 is physically associated with polyribosomes. It is suggested that upon viral infection newly translated viral proteins are primary targets of ISG15. Ubiquitin and other ubiquitin-like proteins such as FAT10 have been shown to target their conjugated proteins for proteasomal degradation thereby feeding antigens into the major histocompatibility complex (MHC) class-I restricted pathway. We hypothesize that ISGylation of viral proteins might as well contribute to proteasomal degradation of these proteins into MHC class I antigen presentation. To elucidate the role of ISG15 conjugation and proteasomal degradation, we treated interferon-induced as well as ISG15 overexpressing cells with the protein synthesis inhibitor *cycloheximid* and were able to show that ISGylation does not target proteins for proteasomal degradation. Thus, the biochemical function of ISG15 conjugation still needs to be further elucidated.

P.D4.02.09

Mechanism of induction of the Toll/Interleukin-1 receptor protein C (TcpC) of uropathogenic *E. coli* (UPEC)

***J. Hemberger**, T. Miethke;*
Universitätsmedizin Mannheim, Mannheim, Germany.

Toll/Interleukin-1 receptor (TIR) proteins are present in many pathogens like uropathogenic *Escherichia coli* (UPEC). They interfere with the TLR-signaling chain, which is an essential part of the innate immune system. Toll/Interleukin-1 receptor protein C (TcpC) from the UPEC strain CFT073 is an essential virulence factor that impairs the innate immune system, i.e. TLRs and inflammasomes, increases the bacterial spread and causes severe organ damage. To find possible gene inductors we tested the native promotor of the operon that includes *tcpC*, a putative promotor directly in front of *tcpC*, and a segment that includes both promotors with *gfpmut2* as a reporter gene that was measured by its fluorescence. We first explored whether different cell culture media influenced the promoter activity. The fluorescence of bacteria in DMEM ceased within 48 h while McCoy and RPMI caused an increase in fluorescence over 72 h. The overall fluorescence strength in McCoy and RPMI cultures was considerably higher as compared to relatively low signals in glucose-minimal-medium and EMEM. Transwell cultures were conducted to investigate, if the induction is dependent on the bacterial density. We could show that an increasing bacterial density dose-dependently induces a higher fluorescence of the putative promotor construct. This effect was stronger when the bacteria were incubated in McCoy medium. We then tested different eukaryotic cell types as possible inductors, since UPECs should recognize them to impair their innate immune response. RAWs did not have an influence on the putative promotor. However, more cells of the urogenital tract have to be tested.

P.D4.02.10

Altered IL-12/IFN-γ pathway in extrapulmonary tuberculosis and visceral leishmaniosis in pediatric patients and related controls

*A. Esteve-Sole[1,2], À. Deyà-Martínez[1,2], A. Noguera-Julian[3], A. Martin-Nalda[4], E. Cobos[4], C. Fortuny[3], P. Soler-Palacin[4], L. Gonzalez-Granado[5,6], C. Gianelli[7], W. Córdova[8], M. Anton[9], V. Bolaño-Doctor[9], A. Sainz-Loyola[10,11], A. Vlagea[9,2], A. Perera-LLuna[10,11], J. Yagüe[9], A. M. Plaza[1], **M. Juan**[9,2], L. Alsina[1,2];*
[1]Pediatric Allergy and Clinical Immunology Department, Hospital Sant Joan de Déu, Institut de Recerca Pediàtrica Hospital Sant Joan de Déu, Esplugues del LLobregat, Spain, [2]Functional Unit of Clinical Immunology Hospital Sant Joan de Déu-Hospital Clinic, Barcelona, Spain, [3]Infectious Diseases Unit, Pediatrics Department; Hospital Sant Joan de Déu, Fundació Sant Joan de Déu-Universitat de Barcelona, Barcelona, Spain, [4]Paediatric Infectious Diseases and Immunodeficiencies Unit, Vall d'Hebron Campus Hospitalari, Barcelona, Spain, [5]Inmunodeficiency Unit, Servicio de Pediatría, Hospital Universitario 12 de Octubre, Madrid, Spain. School of Medicine, Complutense University of Madrid, Madrid, Spain, [6]Instituto de Investigación I+12, Hospital Universitario 12 de Octubre, Madrid, Spain, [7]Immunology Sevice. Hospital Universitario Ramón y Cajal, Madrid, Spain, [8]Centro de referencia nacional de alergia Asma Inmunologia. Instituto Nacional de Salud del Niño, Lima, Peru, [9]Immunology Department. Biomedical Diagnostics Center, Hospital Clinic-IDIBAPS, Universitat de Barcelona, Barcelona, Spain, [10]B2SLab, Departament d'Enginyeria de Sistemes, Automàtica i Informàtica Industrial, Universitat Politècnica de Catalunya, CIBER-BBN, Barcelona, Spain, [11]Department of Biomedical Engineering, Institut de Recerca Pediàtrica Hospital Sant Joan de Déu, Esplugues de Llobregat, Spain.

Genetic defects in IL-12/IFN-γ pathway (known as MSMD, OMIM: 209950) confer increased susceptibility to intracellular pathogens, most commonly mycobacteria. We aimed to define the status of the IL-12/IFN-γ pathway in pediatric patients diagnosed with severe intracellular infections, such as extrapulmonary tuberculosis (TB) or visceral leishmaniasis (VL).
We performed an ambispective cohort study in patients with VL (n=23; mean age 7.42 years old), extrapulmonary TB (n=24, mean age 7.28 years old), and controls (n=41, non-related, n=18 and family-related, n=23). Whole-blood culture in the presence of BCG with or without IL-12p70 or IFN-γ co-stimulation was performed to analyze cytokine secretion. No complete defect of the IL-12/IFN-γ pathway was detected. Yet, patients with VL and extrapulmonary TB showed a statistically significant alteration in IL-12/IFN-γ function, with reduced IFN-γ production and response, respectively. Genetic evaluation is ongoing. Interestingly, related-controls presented a cytokine production pattern closer to that of patients than to that of unrelated-controls.
Pediatric extrapulmonary TB and VL in our media do not seem to be a warning sign for MSMD complete defects. Altered anti-mycobacterial response both in patients and related-controls suggests that the genetic background puts some children at higher risk of these infections. Thorough genetic studies searching for partial or somatic defects in genes related with the IL-12/IFN-γ pathway are needed in these patients.
Study supported by the projects PI12/01990, PI15/01094 and PI13/00676 (Plan Nacional de I+D+I: ISCIII – Subdirección General de Evaluación y Fomento de la Investigación Sanitaria – and FEDER) and the DPI2017-89827-R (Proyectos I+D+i – Programa Retos)

P.D4.02.11

Viral co-option of IFN driven glycolytic programming in infected macrophages

***K. Kotzamanis**[1], J. Edwards-Hicks[1], A. Alghamdi[2], P. Lacaze[1], M. Blanc[1], D. G. Watson[2], A. Finch[1], P. Ghazal[1];*
[1]University of Edinburgh, Edinburgh, United Kingdom, [2]University of Strathclyde, Glasgow, United Kingdom.

Immunity and metabolism have been viewed as separate fields, however, recent evidence show that these two systems are intimately integrated, share resources and cross-regulate each other. Activated immune cells alter their metabolism in order to support effector functions. On the other hand, viruses are obligatory parasites that counter and exploit host pathways, including metabolism in infected cells, to effectively propagate.
The regulation of metabolism in immune cells or virally infected cells has been well studied. However, the precise metabolic regulation that ensues when both immune system and viral infection, in immune cells, interact and compete for the limited resources and available metabolic pathways is not clear. Here, we have sought to investigate this integrative process by studying the metabolic programming of macrophages infected with murine cytomegalovirus (MCMV). Our hypothesis is that productive infection of macrophages by MCMV takes advantage of the early inflammatory metabolomic reprogramming of activated macrophages to establish infection in the cells.
To study this interaction, we have analysed temporally the transcriptome and metabolome of wild type and IFNB-/- or IFNAR-/- macrophages infected with productive and non-productive (attenuated) MCMV strains. This allows unravelling host versus virus directed metabolic alterations observed upon infection of macrophages. We find that cytomegalovirus co-opts early pro-inflammatory changes in glycolysis for establishing infection of macrophages.
This represents a novel previously unappreciated host pathogens interactions pathway.

P.D4.02.12

Murine neutrophils modulate adaptive immunity during brucellosis

***R. Mora-Cartín**[1], C. Gutiérrez-Jiménez[1], A. Alfaro-Alarcón[2], E. Chaves-Olarte[3,1], E. Barquero-Calvo[1], E. Moreno[1];*
[1]Programa de Investigación en Enfermedades Tropicales, Universidad Nacional, Heredia, Costa Rica, [2]Departamento de Patología, Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica, [3]Centro de Investigación en Enfermedades Tropicales, Universidad de Costa Rica, San José, Costa Rica.

Polymorphonuclear neutrophils (PMNs) are part of the first line of defense against microbial pathogens. We have previously demonstrated that PMNs negatively influence the Th1 immune response at early times of *Brucella* infection (PLoS Pathog 9: e1003167). To investigate the influence of PMNs in the adaptive immune response during chronic brucellosis, we removed these leukocytes by means of antibodies against PMNs. during the course of the infection. We have demonstrated that at later times of *Brucella abortus* infection, the bacterium is killed more efficiently in the absence of PMNs than in their presence. Removal of neutrophils during infection decreased spleen inflammation, induced elevated production of INF-γ, IL-6, IL-12 and IL-10, and caused transient cachexia. The absence of PMNs during *Brucella* infection caused a decrease in most antibody isotypes against *B. abortus* LPS. The only exception was the increase of IgG3 isotype, an event linked to the high amounts of IFN-γ produced in neutropenic mice. These results reveal that late removal of PMNs have an unexpected influence in modulating the immune response against brucellosis.

P.D4.02.13

Role of TRPV4 calcium ion channel in immune response against *Mycobacterium tuberculosis* infection

S. K. Naik[1,2], *J. Eich*[2], *M. Hauptmann*[2], *U. Schaible*[2], *A. Sonawane*[1,2,3];
[1]*School of Biotechnology, KIIT University, Bhubaneswar, India,* [2]*Research Centre Borstel, Borstel, Germany,* [3]*Indian Institute of Technology, Indore, India.*

Tuberculosis is caused by the bacteria *Mycobacterium tuberculosis* (Mtb). As a successful human pathogens, Mtb can subvert host defence system and is very difficult to eliminate. Phagosome maturation is essential in innate immune cell effector functions to eliminate bacterial pathogens. Mtb is known to interfere with phagosome maturation through various virulence mechanisms. We found that Mtb infection of macrophages down-regulates expression of TRPV4, a calcium permeable ion channel and member of the Transient Receptor Potential Vanilloid protein family. Initially characterized as an osmolality sensor, TRPV4 can be activated by various physical and chemical stimuli and is involved in phagosome maturation. We found that growth of Mtb in macrophages is increased in the absence of TRPV4, even upon IFN-gamma stimulation when compared to wild type ones. The failure to control mycobacterial growth is likely due to the incapability of IFN-gamma stimulated TRPV4-KO macrophages to drive mycobacteria into phago-lysosomes. Using a pharmaceutical inhibitor in macrophages infected with attenuated mycobacteria resulted in TRPV4 down-regulation of the phagosomal proteins, EEA1 and Rab7 and concomitant failure to promote phagosome maturation thereby leading to increased survival of intracellular mycobacteria. Mycobacterial ESX-1 encoded virulence factors may be involved in TRPV4 modulation as ESX-1 KO strains of Mtb, in contrast to WT ones, failed to down-regulate TRPV4 expression and to promote cell death 48hr post infection. Taken together, our study indicates that TRPV4 calcium ion channel is crucial for the control of Mtb by immune activated macrophages but can be modulated by virulent Mtb in an ESX-1 mediated manner.

P.D4.02.14

Association between NMDA-receptor antibody positivity and infection by herpes virus

I. Nieto-Gañán[1], *P. Walo Delgado*[1], *P. Lapuente Suanzes*[1], *J. Galán Montemayor*[2], *A. Jiménez-Scrig*[3], *Á. Carrasco Sayalero*[1];
[1]*Immunology Department. Hospital Universitario Ramón y Cajal, Madrid, Spain,* [2]*Microbiology Department. Hospital Universitario Ramón y Cajal, Madrid, Spain,* [3]*Neurology Department. Hospital Universitario Ramón y Cajal, Madrid, Spain.*

Introduction: Anti-neuronal antibodies are classified as intracellular and extracellular according to the localization of the antigenic target. Anti-NMDAR antibodies are extracellular antibodies linked to neoplastic disease usually benign ovarian teratoma, with or without encephalitis. They appear occasionally in encephalitis by herpes simplex virus type 1 (HHV1) and enterovirus. The mechanism of this association is not clear. Whether their presence changes the clinical symptoms and prognosis of these encephalitis or if these cases require additional therapy with inmunosupressors is not well known. There are reports of anti-NMDAR antibodies and other herpes virus group encephalitis but they are limited to single case reports.
Material and methods: Retrospective study of anti-NMDAR determined by IFI in neuronal tissues and transfected cells in 39 patients with suspected encephalitis from the Pediatric, adult Neurology and Emergency Departments, which had a positive PCR test for herpes virus not HHV1 (HHV2, VZV, EBV, CMV, HHV6 and HHV7) in CSF.
Results: Two patient tested were positive for anti-NMDAR antibodies in CSF, diagnosed of Handl Syndrome and glioblastoma multiforme and both of them infected by HHV7. Additional 6 patients showed fluorescence in synaptic button.
Conclusions: In addition to HHV1 encephalitis our series reinforce that anti-NMDAR can be present less frequently in other herpes virus infections, with a more complex antigenic pattern than non infectious encephalitis. The presence of fluorescence with nonspecific patterns in neuronal tissues may be due to antigens not described so far. Additional studies are needed in this regard to completely unravel their pathogenic and clinical importance.

P.D4.02.15

Bone marrow contains an expandable reservoir of poly-functional resident-memory CD8$^+$ T cells, which is dependent on IL-15 and Hobit, but not on local antigen recognition

M. Pascutti[1], *S. Geerman*[1], *N. Collins*[2], *G. Brasser*[1], *B. Nota*[3], *R. Stark*[1], *F. M. Behr*[1], *A. E. Oja*[1], *E. Slot*[1], *P. Hombrink*[1], *R. Arens*[4], *L. K. Mackay*[2], *K. P. van Gisbergen*[1], *M. A. Nolte*[1,3];
[1]*Department of Hematopoiesis, Sanquin Research, Amsterdam, Netherlands,* [2]*Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Australia,* [3]*Department of Research Facilities, Sanquin Research, Amsterdam, Netherlands,* [4]*Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, Netherlands.*

The bone marrow (BM) is a large reservoir of memory CD8$^+$ T cells, but it is not clear to what extent these cells belong to the circulatory pool. Using different models of viral and bacterial infections, immunization, cell transfer and parabiosis, we here demonstrate that the BM hosts a population of *bona fide* CD8$^+$ tissue-resident memory T cells (T_{RM}) cells that can develop independently of BM infection or local antigen presentation. BM CD8$^+$ T_{RM} share the transcriptional program of resident lymphoid cells in other tissues, are polyfunctional cytokine producers and depend on IL-15, Blimp-1 and Hobit for their maintenance. They also express a particular set of genes related to tissue remodeling and adhesion that might be induced by the BM environment. Unlike T_{RM} from other tissues, BM T_{RM} reside in the parenchyma, but are also in close connection to the blood stream, due to the high permeability of the BM vasculature. Remarkably, the pool of BM T_{RM} can expand after multiple immunizations with a particular antigen. When mice were sequentially challenged with two different pathogens, T_{RM} against the first pathogen were not deleted to make room for newly formed T_{RM} against the second challenge. These results indicate that BM contains, next to recirculating memory CD8$^+$ T cells, also a unique, functional and expandable pool of cells that take up residency in the tissue. As BM T_{RM} cells develop against numerous pathogens, independently on whether the BM is infected, these findings are highly relevant for successful development of protective vaccination strategies.

P.D4.02.16

Analysis of HCV Core protein molecular associations and its subcellular distribution in Jurkat T cells

C. Fernández Ponce[1], *M. C. Durán-Ruiz*[1], *I. Narbona-Sánchez*[1], ***A. Rodríguez-Moreno***[1], *L. Olvera-Collantes*[1], *J. Niño-Ramírez*[1], *J. P. Muñoz- Miranda*[1], *M. M. Arbulo-Echevarria*[1], *A. Serna-Sanz*[2], *C. Baumann*[2], *R. Litrán*[3], *E. Aguado*[1], *W. Bloch*[4], *F. García-Cózar*[1];
[1]*Department of Biomedicine, Biotechnology and Public Health, University of Cadiz - Institute of Biomedical Research Cadiz (INIBICA), Cadiz, Spain,* [2]*Sciex, Darmstadt, Germany,* [3]*Department of Condensed Matter Physics, University of Cádiz, Puerto Real, Spain., Cadiz, Spain,* [4]*Department of Molecular and Cellular Sport Medicine, Institute of Cardiovascular Research and Sport Medicine, German Sport University Cologne, Cologne, Germany.*

Several viral proteins have been shown to localize in subcellular specific compartments and interact with host molecules, producing changes in the spatial distribution of cellular proteins and inducing alterations in different signaling pathways. HCV Core protein have been described as modulator of biological functions as proliferation, apoptosis, cell cycle, oxidative stress and in CD4+ T lymphocytes, the presence of HCV Core protein induce a regulatory phenotype. Intending to elucidate the mechanisms underlying the cellular alterations caused by this viral protein, we have analyzed HCV Core subcellular localization and its associations with host proteins in Jurkat T cells. We performed immunogold electron microscopy techniques to analyze the subcellular localization of Core protein. Moreover, in order to identify proteins associated with HCV Core, in Jurkat T cells, we carried out pull-down assays combined with Mass Spectrometry Analysis. Thus, in this work, we show the ultrastructural localization of HCV Core in Jurkat T cells and the host molecular interaction networks associated to HCV Core protein.

P.D4.02.17

Early life cytomegalovirus infection

F. Stahl;
University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Congenital infection with cytomegalovirus (CMV) in newborns is the most frequent infectious cause of permanent disabilities. Currently, there is no established therapy available to prevent neonatal CMV disease. Although recognized as a global clinical problem, prenatal factors determining vertical virus transmission from mother to foetus are still unknown. Moreover, it remains poorly understood why most infected neonates remain asymptomatic whereas some show moderate or severe disease symptoms. Here, I aim to investigate factors that could increase the risk of prenatal CMV-infection and severity of neonatal CMV disease. Utilizing human samples in combination with a mouse model for congenital CMV infection I will determine the role of prenatal challenges and the anti-CMV T cell response of mother and child.
Using an established CMV mouse model, I will clarify if maternal stress and medication intake during pregnancy - challenges which are potentially avoidable - render the offspring more prone to develop a severe course of early life CMV infection. In depth characterisation of maternal and neonatal antigen-specific T cells will define their role in control of CMV infection during the early life phase. In summary, I intend to answer pending questions of CMV infection biology at the interface of reproduction, paediatrics, immunology, and virology that could lay the basis for new diagnostic or therapeutic approaches.

P.D4.02.18

Diagnostic potential of rKLO8 and rK28 in discriminating canine visceral leishmaniasis from *Leishmania* vaccinated dogs

*I. E. Pereira[1], L. M. Menegati[1], K. P. Silva[1], M. P. Araujo[2], U. Steinhoff[3], M. S. Duthie[4], **H. C. Teixeira**[1];*
[1]Federal University of Juiz de Fora, Juiz de Fora, Brazil, [2]Santo Agostinho Veterinary Hospital, Belo Horizonte, Brazil, [3]Philipps University of Marburg, Marburg, Germany, [4]Infectious Disease Research Institute, Seattle, United States.

Canine visceral leishmaniasis (CVL) is a major health issue in many tropical and sub-tropical countries and an accurate diagnosis still remains a challenge. The present study aimed to compare the diagnostic accuracy of rKLO8, a new antigenic kinesin-related protein of *Leishmania donovani*, and rK28, a recombinant fusion polyprotein comprising rK26, rK39 and rK9 in detecting CVL. IgG reactivity against *Leishmania braziliensis* whole antigen was also investigated. Sera samples from diseased dogs (CVL, n=44) and healthy endemic controls (EC, n=44) were evaluated for the presence of antigen-specific IgG, IgG1 and IgG2 antibodies by ELISA. The sensitivity and specificity of each ELISA was investigated by ROC curve analysis. Antigen-specific reactivity was also tested against sera from Leish-Tec®-vaccinated dogs. Enhanced levels of IgG and mainly IgG2 to both rKLO8 and rK28 were found in diseased dogs. The ELISAs using rKLO8 and rK28 showed a sensitivity of 77% and 84% for IgG and 78% and 88% for IgG2, respectively. A specificity of 94% for rKLO8 and 95% for rK28 was observed using IgG2 ELISA, and specificity of 100% for both antigens was observed using IgG ELISA. rK28 was the only antigen able to demonstrate differences between vaccinated and infected groups ($p < 0.001$), but showing no difference between vaccinated and control (EC) groups. These results indicate the usefulness of rKLO8 and rK28 in the serodiagnosis of CVL, and suggest that rK28 generates a more accurate diagnosis which can be applied to vaccinated dogs. Financial support: CNPq and FAPEMIG, Brazil.

P.D4.02.19

Complement factor H family proteins associate with severity of bacterial infections in children

***A. E. van Beek**[1,2], N. A. Schweintzger[3], R. B. Pouw[1,2], D. S. Klobassa[3], M. C. Brouwer[1], J. Geissler[4], A. Biebl[5], M. Sagmeister[3], D. Wouters[1], W. Zenz[3], T. W. Kuijpers[2,4], the EUCLIDS consortium;*
[1]Department of Immunopathology, Sanquin Research and Landsteiner Laboratory of the Academic Medical Center, Amsterdam, Netherlands, [2]Departent of Pediatric Hematology, Immunology and Infectious Diseases, Emma Children's Hospital, Academic Medical Center, Amsterdam, Netherlands, [3]Department of General Pediatrics and Adolescent Medicine, Medical University of Graz, Graz, Austria, [4]Department of Blood Cell Research, Sanquin Research and Landsteiner Laboratory of the Academic Medical Center, Amsterdam, Netherlands, [5]Department of Pediatrics, Kepler University Clinic, Medical Faculty of the Johannes Kepler University, Linz, Austria.

Introduction: Complement is part of the innate immune defense against invading pathogens. Concurrent protection from complement is acquired by Factor H (FH), although this protection is thought to be inhibited by FH-related proteins (FHRs). As pathogens recruit FH from human plasma as escape mechanism to increase survival in blood, binding of FHRs instead of FH might prove beneficial for clearing an infection. However, little is known about the plasma levels of these FH family proteins during invasive bacterial infections.
Methods: We included pediatric patients with acute invasive bacterial infections as part of the EUCLIDS study, together with age-matched healthy controls. In-house ELISAs were used to determine FH and FHR plasma levels.
Results: FH levels were low during the acute phase and associated strongly with severity. Similarly, FHR-2 and FHR-4A levels were low in the most severe patients. In contrast, FHR-5 levels were elevated during the acute phase, irrespective of severity or causative microbe, although levels were generally low in patients who suffered from meningococcal disease.
Conclusions: Our study shows that plasma levels of FH family proteins associate with severity of invasive bacterial infections in children. Moreover, we found indications for a novel role for FHR-5 as acute phase protein. Functional in vitro experiments will be needed to further clarify the role of FHR-5. These results highlight a yet unknown role for the FHR proteins in infectious diseases. A better insight into their level and function during invasive infections will help to improve critical care for pediatric patients.

P.D4.02.20

Respiratory Syncytial Virus directly infects natural killer cells and affects anti-viral effector functions

***E. A. van Erp**[1,2], D. Feyaerts[2], M. Duijst[1], L. H. Mulder[1], O. Wicht[1], W. Luytjes[1], G. Ferwerda[2], P. B. van Kasteren[1];*
[1]National Institute for Public Health and the Environment, Bilthoven, Netherlands, [2]Radboud Institute for Molecular Life Sciences, Nijmegen, Netherlands.

Respiratory syncytial virus (RSV) infection can lead to severe respiratory illness and is the main cause of hospitalization in infants under 1 year of age. No vaccines or antivirals are currently available and the determinants of severe disease remain elusive. Natural killer (NK) cells are important effector cells in the anti-viral immune response and likely form a critical component of the early response against RSV infection. NK cells that are recruited to the lung during RSV infection encounter the virus in the presence of (maternal) antibodies. We investigated whether RSV and RSV-antibody complexes affect NK cell functionality, since these cells potentially contribute to immunopathology. We demonstrate for the first time that RSV can directly infect adult and neonatal NK cells. Incubation of RSV with sub-neutralizing antibody concentrations significantly increased the percentage of infected NK cells, and this increase was FcγRIII/CD16 dependent. Upon infection, large numbers of NK cells produced IFN-γ, but this was not accompanied by enhanced killing capacity, as evidenced by the percentage of perforin-secreting cells. RSV-infected NK cells therefore appear geared towards a pro-inflammatory rather than a cytotoxic response. Our findings show that RSV can affect NK cell functionality, an effect that is enhanced in the presence of sub-neutralizing antibody concentrations. Considering that most vaccines that are currently being developed aim at inducing (maternal) antibodies against RSV, it is extremely important to have a good understanding of the possible interactions between innate immune effector cells and virus-specific antibodies and their role in the development of (severe) RSV disease.

P.D4.03 Exploiting host pathogen interaction - Part 3

P.D4.03.01

Characterisation of the effects of interleukin-17A (IL-17A) on toll-like receptor 3 (TLR3)-function and its role in accelerating disease progression in human idiopathic pulmonary fibrosis (IPF)

***M. E. Armstrong**[1], L. Bergin[1], A. N. McElroy[1], G. Cooke[2], P. G. Fallon[1], C. M. Hogaboam[3], N. Hirani[4], S. C. Donnelly[5];*
[1]Trinity Biomedical Sciences Institute, Dublin 2, Ireland, [2]Institute of Technology Tallaght, Dublin 24, Ireland, [3]Cedar-Sinai Medical Centre, Los Angeles, United States, [4]University of Edinburgh, Edinburgh, United Kingdom, [5]Trinity Centre for Health Sciences, Tallaght Hospital, Dublin 24, Ireland.

Introduction: In this study, we investigated the ability of IL-17A to modulate TLR3 function in primary lung fibroblasts from patients with idiopathic pulmonary fibrosis (IPF). Using a case-control study, we also investigated the role of the IL-17A promoter-polymorphism, *IL-17A* G197A (rs2275914) in the development of IPF. Previously, our laboratory demonstrated that defective TLR3 function was associated with a significantly greater risk of mortality and an accelerated rate of decline in lung function, in IPF patients (*AJRCCM*; 188(12):1442-50). We additionally detected increased levels of IL-17A in bronchoalveolar lavage fluid and lung tissue from IPF patients (*PNAS; 111(1):367-72*). Materials and Methods: Primary human lung fibroblasts from IPF patients were treated with Poly(I:C) in the presence or absence of IL-17A. Cytokine, chemokine and type I interferon levels in IPF fibroblasts were determined by ELISA and qPCR, respectively. Patients with IPF were additionally genotyped for the *IL-17A G197A* polymorphism. Results: We established that IL-17A can modulate TLR3-function in IPF lung fibroblasts in order to reduce production of the anti-viral mediators, IFN-β and RANTES. Concomitantly, IL-17A can also synergistically increase TLR3-induced IL-8 from IPF lung fibroblasts. Using a case-control study for IPF, we also demonstrated that individuals who are homozygous for the variant A allele of the *IL-17A* G197A-promoter polymorphism, are significantly more at risk of developing IPF. Conclusions: These results support a novel role for IL-17A in promoting disease progression in IPF via its modulation of TLR3 function in IPF fibroblasts. In addition, this study reveals *IL-17A* G197A as a candidate biomarker in IPF.

P.D4.03.02

In silico identification of *Taenia solium* excretory secretory protein and it's suppressive activity for PI3K/AKT pathway

***N. Arora**, A. Prasad;*
Indian Institute of Technology Mandi, Mandi, Himachal Pradesh, India.

Objective: *Taenia solium* is a parasitic infection of central nervous system, causing neurocysticercosis (NCC). Parasite secretome products modulate host immune response. To understand the cross talk between parasite and human host, excretory secretory (ES)-proteins make up for a suitable target. The objective was to predict *T. solium* ES Protein related with AKT pathway and validate it with LC- MS and wet lab tools. Material & Methods: Cysts were isolated from infected swine and cultured for 24Hrs. The ES-proteins (30ug) was prepared for LC-MS analysis by in-gel tryptic digestion and the protein spectra obtained was annotated. Simultaneously, in silico *T. solium* ES-proteins were predicted using tools TargetP, SignalP, SecretomeP and THMM. Blast2GO was used to perform BlastP, protein annotation and KEGG mapping. After comparing the LC-MS and predicted annotations, PI3K regulatory subunit was identified in both the data sets and investigated the activation of AKT-pathway in response to ES- proteins treatment on human primary macrophages. Results: The *T. solium* proteome consist of ~12000 proteins, ~1000 were predicted as ES-proteins and 355 mapped to global metabolic pathways of which 8 were related with PI3K pathway. LC-MS analysis of in-vitro cultured ES-protein annotated 307 proteins and two were related with PI3K regulation. The cells treated with ES proteins had significantly less activation of pAKT compared to untreated cells. Conclusion: ES proteins are important for immune modulation of host by *T. solium*, and ES-proteins target PI3K/AKT pathway by down regulating it and thus suppressing the immune response.

P.D4.03.03

IMPACT OF ART ON DYNAMICS OF GROWTH FACTORS AND CYTOKINES IN PRIMARY HIV INFECTION

***V. Bordoni**, A. Sacchi, R. Casetti, E. Cimini, C. Pinnetti, A. Mondi, A. Ammassari, A. Antinori, C. Agrati;*
INMI L.Spallanzani, Rome, Italy.

Antiretroviral treatment (ART) of Primary HIV Infection (PHI) has demonstrated virological and immunological benefits. The effect of early ART during PHI on the level of growth factors and chemokines modulating immune cell functions remain to be established. The aim of our work was to analyze the dynamics of 27 cytokines (pro-inflammatory, Th1, Th2), chemokines (IL-8, CCL27, CXCL9, MCP-1, MIP-1b, RANTES) and growth/regulation factors (TRAIL, SCF, SCGF-b, HGF, M-CSF, G-CSF, GM-CSF, LIF) in plasma of HIV infected patients treated during PHI.Patients with PHI (n=43) were enrolled before, 24 and 48 weeks after therapy initiation. Quantification of soluble immune mediators described above was evaluated in HIV infected patients and healthy donors (HD, n=9) by Luminex technology . The cytokines profile was strongly perturbed in primary HIV infected patients when compared to HD. After 48 weeks of ART, some of these factors were restored to HD level (IL-7, IL-9, LIF) while others persisted higher than HD (IL-6, IL-10, G-CSF). Interestingly, a subset of chemokines, such as MCP-1, IL-8, MIP-1b, RANTES and CCL27, as well growth factors such as HGF, SCF and GM-CSF, increased during ART reaching values significantly higher than HD after 48 weeks. The increase of chemokines with antiviral activity and of growth factors with hematopoietic and immunomodulatory properties may have a beneficial effect. Other studies are mandatory to evaluate the long lasting levels of these factors to clarify their possible role in the context of protection/pathogenesis.

P.D4.03.04

Stimulation of MAC-inhibitory protein (CD59) but no complement decay-accelerating factor (CD55) induces release of neutrophil extracellular trap (NETs)

***O. Ciepiela**, W. Bystrzycka, J. Holka, P. Tarnowska, A. Manda-Handzlik, M. Wachowska;*
Medical University of Warsaw, Warsaw, Poland.

Introduction: MAC-inhibitory protein, also known as CD59 is a membrane inhibitor of reactive lysis found on cell surface of leukocytes and erythrocytes. When complement activation leads to deposition of C5b678 on host cells, CD59 can prevent C9 from polymerizing and forming the complement membrane attack complex. CD55 recognizes C4b and C3b fragments that are created during activation of C4 (classical or lectin pathway) or C3 (alternative pathway) thus indirectly blocks the formation of the membrane attack complex. CD59 and CD55 attach to host cells via a glycophosphatidylinositol (GPI) anchor. A mutation of PIG-A gene, which leads to deficiency of GPI, is found in patient with paroxysmal nocturnal hemoglobinuria. The aim of the study was to establish, if release of neutrophil extracellular traps might be dependent on CD59 or CD55 activation. Materials and methods: Neutrophils were isolated from the blood of healthy donors. Cells were obtained by density gradient centrifugation and subsequent polyvinyl alcohol sedimentation of erythrocytes. Subsequently, neutrophils were incubated with human antiCD59 or antiCD55 monoclonal antibody and 100 nM PMA was added to stimulate NETs release. The process of NETs release was assessed 3h post stimulation by fluorescent microscopy and fluorometry. Results: Anti-CD59 antibody induced NETs release in a concentration-dependent manner, with the highest release at 0.25 - 1.25 µg/ml. Fluorescent microscopy confirmed results of quantification method. None of studied anti-CD55 monoclonal antibody concentration lead to release of NETs. Conclusion: Neutrophil extracellular traps release is dependent on activity of GPI-anchored proteins which regulates complement activation pathway.

P.D4.03.05

The role of Fas/FasL receptors in pathogenesis of the inflammation of the nervous system induced by HSV-1 and 2 infection

***M. Krzyzowska**[1], A. Kowalczyk[1], K. Eriksson[2];*
[1]Wroclaw Research Centre EIT+, Wroclaw, Poland, [2]University of Gothenburg, Department of Rheumatology and Inflammation Research, Gothenburg, Sweden.

Introduction. The aim of this project was to determine the role of Fas/FasL receptor signalling in pathogenesis of the inflammatory lesions occurring during herpes simplex virus type 1 and 2 (HSV-1/2) infection of the central and peripheral nervous system. Materials and methods. In vitro models consisted of mixed glial culture, primary micgroglia culture obtained from C57BL6 mice. In vivo studies used intranasal model of C57BL6 mice infected with HSV-1 McKrae strain, and genital model of C57BL6 mice infected with HSV-2 333 strain. Apoptosis, Fas and FasL expression and phenotype of immune competent cells were accessed using confocal microscopy and flow cytometry. Results. Upon HSV-1 or HSV-2infection, microglia underwent early apoptosis and up-regulated FasL expression, while HSV-1/2 infected astrocytes also up-regulated FasL and were resistant apoptosis. Both microglia and astocytes were resistant to Fas-induced apoptosis. However, Fas- stimulated microglia became M1-type cells and switched the profile of produced cytokines. In vivo, FasL expression was detected on astrocytes surrounding infected sites in spinal cord (HSV-2) and brain (HSV-1) as well as on microglia within glia limitans and infected neuronal tissue. Fas positive cells were mostly infiltrating lymphoidal cells. Conclusion. We found that there is a correlation between HSV infection and reaction of cells to Fas/FasL induced apoptosis. Fas/FasL signalling can participate both in direct elimination of HSV infection, but also in a complex regulation of the local inflammatory response and mounting of the specific anti-viral response through non-apoptotic signalling. This work was supported by 2015/18/M/NZ6/00414 grant.

P.D4.03.06

Lack of Polyfunctional Cytomegalovirus-specific T cells in Hemodialysis Patients

***F. Lay**[1], K. Shu[1], I. Chen[1], J. Chia[2], Y. Chiu[1,2];*
[1]Far Eastern Memorial Hospital, New Taipei City, Taiwan, [2]National Taiwan University, Taipei, Taiwan.

Background: Polyfunctional T cells are critical for maintaining protection against pathogens. Patients with end-stage renal disease (ESRD) are at increased risks for infectious complications and their T cell immunity against viruses may be impaired. The current study intends to investigate T cell immunity in ESRD patients by analyzing T cell differentiation and polyfunctionality against cytomegalovirus (CMV), an ubiquitous pathogen. Method: 21 healthy individuals, 13 patients with chronic kidney disease (CKD) and 47 ESRD patients were enrolled in this study. All donors were seropositive for CMV.
Two CMV peptide pools (IE1 and pp65) were used to stimulate PBMCs and four effector functions were measured by multicolor flow cytometry (IL-2, TNFα, IFNγ and CD107a) to identify polyfunctional cells. Result: Age of the three groups was similar (mean, 60 years old). Patients with renal disease, especially ESRD patients, showed increase levels of T cell differentiation, including the increase in $CD4^+$ and $CD8^+$ TEM and TEMRA cells. While the cellular frequencies of CMV-reactive $CD4^+$and $CD8^+$T cell were comparable, polyfunctionality were dramatically reduced in ESRD. Among healthy individuals, CKD and ESRD patients, CD4+ CMV-IE1-reactive polyfunctional cell frequencies were 2.0%, 2.2% and 0%, respectively (p=0.002). CD8+ CMV-pp65-reactive polyfunctional cell frequencies were 12.4%, 8.8% and 0.8% in the three groups (p<0.001). The impairment of polyfunctionality correlated with increased PD-1 expression. Conclusion: ESRD patients are characterized by the loss of polyfunctional, CMV-specific T cells and increased PD-1 expression. Whether impaired CMV-specific polyfunctionality could be reversed by PD-1 blockade requires further study.

P.D4.03.07

Crosstalk of autophagy and reactive oxygen species synthesis in reactive nitrogen species-induced neutrophil extracellular traps formation

***A. Manda-Handzlik**[1,2], M. Wachowska[1], W. Bystrzycka[1,2], O. Ciepiela[1], U. Demkow[1];*
[1]Medical University of Warsaw, Warsaw, Poland, [2]Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland.

Autophagy is a natural, self-degradative process which regulates neutrophil antimicrobial functions. One of the neutrophils' antimicrobial strategies is the formation of neutrophil extracellular traps (NETs). Although mechanisms of NETs formation are extensively studied, some aspects of this process, e.g. the role of reactive nitrogen species (RNS), remain poorly understood. Our aim was to investigate RNS as NETs stimuli and to identify the role of autophagy in this process.
Human blood neutrophils were stimulated with nitric oxide (NO) donor - SNAP (S-Nitroso-N-acetylpenicillamine), peroxynitrite or phorbol 12- myristate 13-acetate (PMA). NETs release and the synthesis of reactive oxygen species (ROS) were assessed by fluorometry and fluorescent or light microscopy. Autophagy was analyzed by western blotting as accumulation of LC3-II protein.
We found that contrary to PMA, RNS stimulate NETs release without accumulation of LC3-II. Inhibitors of class III PI3 kinases (3-methyladenine, 3-MA, and wortmannin, used as inhibitors of autophagy), but not inhibitors of the autolysosome formation, drastically reduced NETs formation upon RNS treatment. RNS only slightly increased ROS production by neutrophils, but 3-MA and wortmannin significantly decreased ROS production by RNS-stimulated neutrophils. Finally, we found that activity of NADPH oxidase was necessary for SNAP-induced NETs release and contributed to peroxynitrite-induced NETs formation.
We conclude that RNS efficiently stimulate NETs formation and PI3K activity, but not autophagic flux, is necessary for this process. PI3K influences NETs formation via regulation of NADPH oxidase activity.
Acknowledgements
This work was supported by the National Science Centre, Poland (Preludium 2015/19/N/NZ6/01317) and the Foundation for Polish Science (POWROTY/2016-2/7).

P.D4.03.08

Functional roles of atypical IkB family members in macrophages

A. Matthies[1,2]*, K. Katsoulis-Dimitriou*[1,2]*, C. Plaza-Sirvent*[1,2]*, I. Schmitz*[1,2]*;*
[1]*Helmholtz Centre for Infection Research, Braunschweig, Germany,* [2]*Otto von Guericke-University, Magdeburg, Germany.*

The activation of the transcription factor NF-κB is regulated by inhibitor of NF-κB proteins (IκB proteins), which include not only classical cytoplasmic proteins such as IκBα, but also atypical nuclear proteins such as Bcl-3, IκBζ and $I\kappa B_{NS}$. Notably, the functions of the atypical IκB proteins in macrophages as well as their interactions remain poorly understood. To address this, we tested whether the atypical IκB proteins Bcl-3, IκBζ and $I\kappa B_{NS}$ in macrophages exhibit interdependent regulation of their expression and function. Our results showed that the atypical IκB proteins are expressed in primary bone-marrow-derived macrophages by Toll-like agonist with different kinetics. Interestingly, following LPS stimulation of RAW 264.7 macrophages, IκBζ and $I\kappa B_{NS}$ are upregulated early on, whereas Bcl-3 is upregulated at later time points. To analyze the functional relevance of IκB expression in macrophages, we infected wildtype and IκB-deficient macrophages with GFP-expressing *Staphylococcus aureus in vitro*. In preliminary experiments, we detected no alterations of intracellular bacteria in IκB-deficient macrophages compared to wildtype controls suggesting that IκB deficiency does not affect the phagocytosis capacity of macrophages. Furthermore, we found no difference in proliferation of intracellular *S. aureus* of IκB-deficient macrophages revealing that IκB deficiency does not alter the killing activity of macrophages. To extend this analysis also in *in vivo* experiments, we generated LysM-Cre,$I\kappa B_{NS}^{flox/flox}$ mice that lack $I\kappa B_{NS}$ specifically in macrophages and neutrophils. An initial immune phenotyping revealed no alterations in frequencies or absolute numbers of macrophages, neutrophils and dendritic cells suggesting that $I\kappa B_{NS}$ is dispensable for the development of these cells.

P.D4.03.09

Modulation of T-cell cytokine profiles by (killer) B-cells during Tuberculosis disease

D. K. Moore*, I. C. Van Rensburg, A. G. Loxton;*
Stellenbosch University, Cape Town, South Africa.

Rationale: Emerging evidence has implicated B-cells as important players in the defense against *Mycobacterium tuberculosis*. This study aimed to identify potential mechanisms by which B-cells may modulate T-cell functioning, as they have been regarded as the main immune cells involved in eradicating TB disease. **Method:** T-cells were cultured with BCG-exposed or naive B cells that had been pulsed with or without CD40L and IL5, to induce a regulatory B-cell subtype. These killer B_{regs} cell function in TB disease is still unclear. **Results:** Killer B_{reg} frequencies are elevated in Mtb unexposed healthy individuals when compared to healthy exposed individuals, however this difference was not significant but similar to studies that have found an increase in killer B_{reg} frequencies following successful TB treatment. This suggests a role of B-cells in protective immune responses to TB disease. An increase in the frequency of effector T-cells, although non-significant, was observed in healthy Mtb exposed individuals following cultured with unstimulated B-cells and a decrease following culture with BCG-stimulated B-cells. This may imply a role of B-cells in T-cell functioning through regulation of phenotypic frequencies. Finally, B-cells that were CD40L/IL-5 pulsed or not induced cytokine production by both CD4 and CD8 T-cells in healthy exposed and unexposed individuals. However, these alterations in cytokine profiles were not significant. **Conclusion:** B-cells and killer B_{regs} influence T-cell behavior by modulating phenotype development and cytokine secretion. These results suggest a key role of B_{regs} in initiating and guiding the immune response against Mtb.

P.D4.03.10

Multiparameter analysis of association between host immune reactivity and pulmonary tuberculosis activity

I. Y. Nikitina*, A. V. Panteleev, T. A. Nenasheva, T. R. Bagdasarian, V. A. Shorokhova, R. B. Amansahedov, I. V. Lyadova;*
Central Tuberculosis Research Institute, Moscow, Russian Federation.

Tuberculosis (TB) is a highly heterogeneous infectious disease characterized by different outcomes. Both impaired and exacerbated immune responses contribute to TB pathogenesis. However, the exact role of immunological hypo- and hyper-reactivity in TB pathology and their biomarkers are unknown. Here we analyzed how various indicators of innate and adaptive immunity are associated with TB severity.
Sixty four TB patients (TBP) and 28 healthy contacts (HC) were included in the study. In each participant, we performed QuantiFERON-TB Gold Plus assay and analyzed 46 immune factors in serum and antigen-induced plasma using xMAP multiplex assay. In TBP, the degree of pulmonary destruction, TB extent, systemic intoxication and bacteria excretion were accurately evaluated.
TBP and HC could be well discriminated based on the levels of IL-2, IFN-γ, IP-10 and IL-8 in antigen-induced plasma ($p<0.005$) and GM-CSF in serum ($p<0.0001$). Analysis of immunological analytes in antigen-induced plasma of TBP identified 14 factors that discriminated TBP into immunologically “hypo-” and “hyper-reactive” groups. The factors included type I and III interferons, members of IL-10 and IL-12 cytokine superfamilies. Based on the level of IP-10 in serum, TBP clustered into other two groups. High IP-10 levels (>730 pg/ml) were indicative of severe pulmonary destruction ($p<0.05$) and predicted its slow/unfavorable dynamics.
In conclusion, we identified factors that discriminated TBP into groups with high and low immune reactivity, which may be helpful for personalized implementation of pathogenetic therapy. IP-10 can serve as a candidate biomarker of TB infection activity and patients’ responsiveness to anti-TB treatment.
Supported by Grant RSF-17-75-10197

P.D4.03.11

Evaluation of interferon-gamma role during zika virus murine experimental infection

C. M. Polonio*, N. Zanluqui, L. Oliveira, C. Longo, J. Peron;*
Neuroimmune Interactions Laboratory - Department of Immunology - University of São Paulo, São Paulo, Brazil.

The flavivirus Zika (ZIKV) was recently introduced in Brazil causing an alarming increase of babies born with microcephaly. Genetic differences, mainly related to Interferons (IFN), may greatly influence the susceptibility to infection. IFNs are classified in Type I – III, a major cytokine family involved in antiviral and anti-tumoral immune response. IFN-γ is a Type II IFN mainly produced by T lymphocytes and NK cells. Interestingly it is known that during DENV infection, also a flavivirus, IFN-γ-dependent mechanisms were associated with DENV resistance. Thus, we evaluated the IFN-γ role during ZIKV infection. We infected WT and IFN-$\gamma^{-/-}$ C57BL/6 mice with $1x10^2$ PFU intravenously and analyses were performed 1 and 3 days post infection. Our results demonstrated an important role of the IFN-γ in suppressing ZIKV infection, since IFN-$\gamma^{-/-}$ mice had higher viral load at spleen 1 and 3 days post infection when compared to controls.
In addition, we observed an increase in type I IFN cytokines production on the first day of infection in the WT mice, followed by a decrease, unlike the deficient animals, which production peaked on day 3. Interestingly, the same was observed for the expression of the IRF7 and IRF9 transcription factors, as well as for interferon-induced gene expression. Taken together, our data demonstrate the importance of the IFN-γ cytokine during the initial antiviral response against ZIKV, as the expression of factors responsible for this response were somehow delayed.

P.D4.03.12

Taenia solium **cyst’s excretory/secretory (ES) proteins suppress Th1 immune response in human macrophages**

A. Prasad*, N. Arora;*
School of Basic Sciences, Indian Institute of Technology Mandi, Mandi, HP, India.

Objective: Larvae of *Taenia solium*cause Neurocysticercosis (NCC), which is most widespread cause of acquired epilepsy in developing countries. Excretory/secretory (ES) proteins released by larvae of *T. solium* are crucial for parasite survival and represent potential targets for novel intervention strategies. The current study was carried out to immune characterise ES proteins of *T. solium*. **Method**: Cysts were isolated from naturally infected pork muscles and cultured in RPMI-1640 complete media for 24 hours. ES proteins were characterized by silver staining, 1D-NMR, LC-MS spectroscopy and enzyme electro immune transfer blot (EITB) with NCC patients serum. Human macrophages isolated from buffy coat were stimulated with the ES proteins for 24hrs to look for their immune cell stimulating capabilities. **Results**: NMR spectra showed a number of metabolites being excreted by the cyst. We identified several bands of <50kDa on EITB. LC-MS analysis of in vitro cultured ES protein annotated 307 proteins and two were related with PI3K regulation.QPCR and ELISA had shown significantly low IL6, IL1βand enhanced IL4 cytokines expression. **Conclusion**: The ES proteins of *T. solium*suppress the Th1 immune response and help in parasite survival in host.

P.D4.03.13

Complement factor H-related protein 1 impairs factor H acquisition during complement evasion by the malaria parasite *Plasmodium falciparum*

T. Reiß[1], *T. F. Rosa*[1], *R. P. Bobbert*[1], *P. F. Zipfel*[2], *C. Skerka*[2], *G. Pradel*[1];
[1]*RWTH Aachen University, Aachen, Germany,* [2]*Hans-Knöll-Institute, Jena, Germany.*

Human complement is the first defense line against invading pathogens, including the unicellular malaria parasite *Plasmodium falciparum*. We previously demonstrated that human complement represents a particular threat for the clinically relevant blood stages of the parasite. To evade complement-mediated destruction, these acquire factor H (FH) via specific receptors, resulting in inactivation of complement factor C3b. We now report that the FH-related protein FHR-1 competes with FH for binding to the malaria parasite. FHR-1, which is composed of five short consensus repeat domains with variable homology to FH but which lacks the C3b regulatory activity, accumulates on the surfaces of the intraerythrocytic schizonts as well as of free merozoites. While binding of FH to schizont-infected red blood cells is increased in FHR-1-deficient human serum, the addition of recombinant FHR-1 decreases FH-binding and in consequence parasite viability. We conclude that FHR-1 acts as a modulator of human immunity by counteracting FH-mediated microbial complement evasion.
This work was funded by grants PR905/8-2 and PR905/12-1 of the Deutsche Forschungsgemeinschaft (to GP and CS). TFAR received a fellowship from the Science without Borders Foundation Programme CAPES.

P.D4.03.14

Immune regulation by myeloid cells leads to protection of *Plasmodium berghei* ANKA-infected Ifnar ko mice from experimental cerebral malaria

P. J. Korir[1], *L. M. Jenster*[1], *J. M. Kuepper*[1], *J. F. Scheunemann*[1], *J. J. Reichwald*[1], *A. Mueller*[2], *A. Hoerauf*[1,3], *D. R. Engel*[4], ***B. Schumak***[1];
[1]*Institute of Medical Microbiology, Immunology and Parasitology, University of Bonn, Bonn, Germany,* [2]*Centre of Infectious Diseases, Parasitology Unit, Heidelberg University Hospital, Heidelberg, Germany,* [3]*DZIF, Partner Site Bonn-Cologne, Germany,* [4]*Department of Immunodynamics, University Essen Duisburg, Essen, Germany.*

Inflammatory responses aim at pathogen elimination but need tight control as excessive immune activation can cause severe host-induced pathology. Cerebral malaria is a fatal complication of Plasmodium ssp. infection and an important example for overwhelming Th1-driven inflammation. This disease can be studied with the help of experimental models. Whereas wildtype (WT) mice develop experimental cerebral malaria (ECM) upon infection with *Plasmodium berghei* ANKA (PbA), transgenic mice that lack type I interferon receptor (*Ifnar*) dependent signalling are protected from ECM. Using transgenic *Plasmodium berghei* ANKA parasites expressing ovalbumin (PbA-OVA), we show that ECM-protected IFNAR ko mice did not differ in their antigen-specific cytotoxic T cell responses compared to infected WT mice suffering from ECM. Importantly, spleens of ECM-negative *Ifnar1*$^{-/-}$ mice but not of ECM-positive WT mice contained increased numbers of both CD8^{+}T cells and distinct myeloid cells, including M2 macrophages, confirmed by expression of typical markers such as RELMα and YM1 as well as their function shown by elevated arginase activity. Furthermore, *in vitro* coculture experiments demonstrated that individual myeloid cells derived from bone marrow were capable to exhibit suppressive capacities. We conclude that type I IFN signalling is not required for the generation of antigen-specific T cells in *PbA*-infected *Ifnar1*$^{-/-}$ mice but its lack rather results in the successful induction of immune regulatory pathways driven by myeloid cells that control CD8 T cells resulting in protection from ECM.
Funding: Jürgen-Manchot-Stiftung (PhD scholarship J.F.S., J.J.R.), BONFOR (J.F.S., B.S.), EXC1023 (J.F.S., CC, A.H., B.S.)

P.D4.03.15

Combined IL17 and IL22 secretion profile characterizes the efficacy of MTB response

Y. Todorova[1], *R. Emilova*[1], *V. Milanov*[2], *M. Zamfirova*[3], *T. Varleva*[3], *M. Nikolova*[1];
[1]*National Reference Laboratory of Immunology, National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria,* [2]*Clinic of Phthisiatry, Multiprofile Hospital for Active Treatment of Lung Diseases "St. Sofia", Sofia, Bulgaria,* [3]*Programme "Improving the sustainability of the National Tuberculosis Programme", Ministry of Health, Sofia, Bulgaria.*

Tuberculosis remains a major cause of death and morbidity worldwide. Current interferon-γ release assays for diagnosis of MTB infection do not predict its clinical course and the need of specific therapy. The mechanisms of protective MTB-specific immune response have not been clarified. IL-17 and IL-22 were pointed out as key players, but data from human studies remain contradictory. Aim: To characterize IL-17 and IL-22 secretion profiles in subjects with different efficacy of MTB-specific immune response. Materials and methods:Peripheral blood samples from: A.QFT(-) healthcare workers in intensive contact with MTB (n=11); B.QFT(+)healthy persons (LTBI, n=21), C.QFT(+) active TB patients (ATB, n=12) were analyzed. IL-17 and IL-22 production was determined after 18h stimulation with phytohemagglutinin (A-C) or CD4- and CD8-specific MTB peptides (Quiagen) (B,C) by ELISA (Affymetrix, eBioscience). Results: Non-specific stimulation revealed significantly decreased IL-17 and IL-22 secretion potential in ATB as compared to individuals with efficient response to MTB (A,B): (mean pg/ml): 71vs.204 and 190 ($p<0.05$), and 272vs.719 and 1027 ($p<0.05$). Efficient containment of MTB in LTBI was associated with domination of IL-17+ and IL-22+CD8 T while increase of IL-17+CD4 and decrease of IL-22+CD8 was observed in ATB. Finally, high level of MTB- stimulated IFN-γ (>5 IU/ml) in LTBI was combined with significantly increased level of CD8 IL-22+ MTB-specific CD8 (162vs.77, $p<0.05$). Our data suggest that while both IL-17 and IL-22-CD8 T are engaged in the protective response to MTB, the level of IL-22+CD8 may be determinant for containment of latent infection. Supported by research grant No13-1/14.12.2017, Bulgarian National Science Fund.

P.D4.03.16

Effect of probiotics on cytokines gene expression in gingival epithelial cells challenged with *Porphyromonas gingivalis*

G. C. Vale[1,2], *E. S. Ando-Suguimoto*[1], *E. Albuquerque-Souza*[1], *M. P. Mayer*[1];
[1]*University of São Paulo, São Paulo, Brazil,* [2]*Federal University of Piauí, Teresina, Brazil.*

Although there is a vast amount of data supporting the pivotal role for cytokines in mediating the host response to periodontal pathogens and the associated tissue damage, literature is scarce regarding the mechanisms underlying the beneficial effect of probiotics. Thus, this study aimed to evaluate the effect of probiotics on gene expression of cytokines by gingival epithelial cells (GECs) challenged with *P. gingivalis.* OBA-9 GECs (~2,5x 10^5cells/well) were challenged with *P. gingivalis* strain (W83 or ATCC 33277) and co-infected with two tested probiotic strains (*L. rhamnosus* and *L. acidophilus*) at a multiplicity of infection (MOI) of 1:1,000 for 2h. OBA-9 viability was measured by trypan blue exclusion assay. Levels of gene expression encoding cytokines (IL-1β, IL-8, IL-6, IL-18, TNFα) were evaluated by RT-qPCR. *P. gingivalis* challenge with both strains resulted in a significant loss of OBA-09 cells viability, which was partially reversed by the use of both probiotics. *P. gingivalis* W83 or ATCC 33277 promoted an increase in the transcription of pro-IL1-β, IL-8, and TNFα when compared to control no infected cells (OBA). The addition of both probiotics to *P. gingivalis* challenged cells resulted in decreased production of IL-1β and TNFα. Furthermore, challenged OBA-9 cells showed decreased expression of IL-18, which was further decreased by co-infection with the probiotics. Overall, the two probiotics have induced an altered cytokines expression profile, regarding the transcription of other inflammatory mediators such as IL-6 and IL-8. In conclusion, the probiotics tested seems to have a direct immunomodulatory effect on gingival epithelial cells.

P.D4.03.17

CD300a expression on T lymphocytes from naïve HIV-1 infected patients for cART is associated to good prognosis and a higher polyfunctional HIV-specific CD8+ T cell response

J. Vitallé[1], *I. Terrén*[1], *L. Gamboa*[1], *A. Orrantia*[1], *L. Tarancón-Díez*[2], *M. Genebat*[2], *E. Ruiz-Mateos*[2], *M. Leal*[2], *S. García-Obregón*[1], *O. Zenarruzabeitia*[1], *F. Borrego*[1,3,4];
[1]*BioCruces Health Research Institute, Barakaldo, Spain,* [2]*Institute of Biomedicine of Seville (IBiS), Virgen del Rocío University Hospital, Seville, Spain,* [3]*Ikerbasque, Basque Foundation for Science, Bilbao, Spain,* [4]*Basque Center for Transfusion and Human Tissues, Galdakao, Spain.*

CD300a inhibitory receptor, in healthy donors, is differentially expressed on CD8+ and CD4+ T cell subsets. CD300a expression identifies a population that is highly polyfunctional on Th1 cells and it has been related to a higher cytotoxic capacity on CD8+ T lymphocytes. Importantly, this receptor is known to be involved in viral mechanisms to enter host cells and to escape from immune system attack. In this study, we observed a differential expression of CD300a on CD8+ and CD4+ T cell subsets from naïve chronically HIV-1 infected patients for combined antiretroviral treatment (cART) and patients on suppressive cART, in comparison with healthy donors. Moreover, we found a negative correlation of CD300a expression on T cells with markers of HIV-1 infection progression, only in untreated patients. Finally, we stimulated CD8+ T lymphocytes from naïve patients for cART with a pool of HIV-1 peptides during 6 hours and we measured the degranulation (CD107a) and the IFNγ, TNFα and MIP-1β production. We observed a higher polyfunctional HIV-specific response in cells expressing CD300a than in cells negative for the receptor. Our results suggest that CD300a could have an important role on T cell regulation during chronic HIV-1 infection and reveal the potential applicability of this molecule as a biomarker for the prognosis of these patients. Funding: Plan Estatal de I+D+I 2013-2016, ISCIII-Subdirección de Evaluación y Fomento de la Investigación-Fondo Europeo de Desarrollo Regional (FEDER) (PI13/00889) and Marie Curie Actions, Career Integration Grant, European Commission (CIG 631674).

P.D4.03.18

IL-18, IL-18BP, IL-37 and IP-10 levels in severe and non-severe cases of pulmonary tuberculosis (TB)

S. Wawrocki[1], *G. Kielnierowski*[2], *M. Druszczynska*[1];
[1]*Division of Cellular Immunology, Department of Immunology and Infectious Biology, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland,* [2]*Regional Center Hospital for Tuberculosis Lung Diseases and Rehabilitation in Tuszyn, Tuszyn, Poland.*

Introduction: The identification of improved markers of pulmonary TB severity has been recognized as a key research area. The development of active TB depends on the strength of immune-driven mechanisms involving the chain of multiple cytokines and chemokines including interleukin 18 (IL-18), IL-18 binding protein (IL-18BP), IL-37 and Interferon γ-induced protein 10 (IP-10). Aim: The aim of the study was to compare the serum and *M.tb* -stimulated whole blood levels of IL-18, IL-18BP, IL-37 and IP-10 in pulmonary TB patients with different disease severity.

Materials and Methods: Serum and *M.tb*-stimulated whole blood cultures were obtained from 95 adult individuals with active pulmonary TB classified into two groups according to the extent and type of chest radiograph findings. The studied proteins in sera and plasma were determined immunoenzymatically (DuoSet®ELISA, R&D). Results: The results showed the similar levels of IL-18, IL-37 and IP-10 in patients with severe and non-severe forms of TB. Significantly higher levels of IL-18BP in *M.tb*-stimulated cultures of blood from severe TB cases compared with non-severe patients were noticed (p=0,04). The levels of IL-18BP were correlated with the concentration of IL-18 in such cultures . Severe TB cases were characterized by increased ratio of IL-18BP/IL-37 and IL-18/IL-37 in serum as well as IL-18BP/IL-37 and IL18BP/free IL-18 in *M.tb*-stimulated cultures. Conclusion: The ratio of IL18BP/IL-37 measured in both serum and M.tb-stimulated cultures may serve for distinguishing severe TB forms from non-severe. This work was supported by the National Science Centre Grant no. 2015/19/N/NZ6/01385 and 2016/21/B/NZ7/01771.

P.D4.04 Exploiting host pathogen interaction - Part 4

P.D4.04.01

The translation elongation factor-1 alpha (Tef1) of *Candida albicans* modulates mouse CD4+ T cell responses *in vitro*

K. Alberter[1], *P. Dasari*[2], *P. Zipfel*[3], *N. Beyersdorf*[1];
[1]*University of Würzburg, Institute for Virology and Immunobiology, Würzburg, Germany,* [2]*Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Jena, Germany,* [3]*Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Friedrich Schiller University, Jena, Germany.*

Invasive infections with the saprophytic yeast *Candida albicans* are a major cause of morbidity in immunocompromised patients. While the interaction of cells and molecules of innate immunity with *C. albicans* has been studied to great depth, comparatively little is known about the modulation of adaptive immunity by *C. albicans*. In particular, direct interaction of proteins secreted by *C. albicans* with $CD4^+$ T cells has not been studied extensively. We here report that the translation elongation factor-1 alpha (Tef1), which is secreted by *C. albicans,* binds to mouse $CD4^+$ T cells and to a lower degree also to mouse B and $CD8^+$ T cells. Functionally, purified Tef1 recombinantly expressed in *Pichia pastoris* enhanced IFNγ and IL-17 secretion by anti-CD3 monoclonal antibody-stimulated splenocytes identifying it as a factor inducing pro-inflammatory cytokine secretion. Our preliminary data further indicate that $CD4^+$ $Foxp3^+$ $CD25^+$ regulatory T cells (Treg) bind Tef1 much better than $CD4^+$ $Foxp3^-$ $CD25^-$ conventional $CD4^+$ T cells. We assume that binding of Tef1 to Treg might 'neutralize' its activity as IFNγ secretion upon anti-CD3 monoclonal antibody and Tef1 stimulation was enhanced in the absence of Treg. The interaction of Tef1 and Treg might, thus, contribute to the commensalism of *C. albicans* and might be an important pathway protecting the organism form overshooting pro-inflammatory immune responses in invasive or even septic candidiasis. This study was funded by a grant from the DFG (CRC124 FungiNet - project C6).

P.D4.04.02

An impact of holo-transferrin on release of Neutrophils Extracellular Traps (NETs)

W. Bystrzycka[1,2], *A. Manda-Handzlik*[1,2], *M. Wachowska*[1], *U. Demkow*[1], *O. Ciepiela*[1];
[1]*Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Medical University of Warsaw, Warszawa, Poland,* [2]*Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland.*

Metal ions, were reported to be found in the structure of Neutrophil Extracellular Traps (NETs). However, to this end, little is known about the exact functions of these ions in NETs formation. Taking into account the invaluable role of microelements in innate immunity, an in-depth understanding of the impact of metal ions on NETs release is desirable.
The aim of this study was to investigate the impact of human holo-transferrin (hT) on the release of NETs.
Neutrophils were isolated from the blood of healthy donors using density gradient centrifugation method and incubated with hT. PMA or calcium ionophore CI were added to stimulate NETs release. The process of NETs release was assessed 3h post stimulation by fluorescent microscopy and fluorometry. Intracellular production of reactive oxygen species (ROS) was assessed by fluorometrical analysis using dihydrorhodamine 123 (DHR123). Nitro blue tetrazolium (NBT) reduction assay was performed to determine how much superoxide is produced.
The delivery of hT to neutrophils contributed to an inhibition of netosis after stimulation with PMA. Transferrin at concentration of 5 µg/ml significantly decreased the amount of released extracellular DNA. Analysis of oxidative burst by DHR oxidation and NBT reduction assay exposed that hT does not affect ROS release. Moreover, hT did not affect NADPH-oxidase-independent NETs release.
Iron is a microelement involved in the formation of NETs. Further studies focusing on the mechanism in which hT affects netosis are of great importance.
This study was supported by funding from the National Science Centre, Poland; Preludium grant no. 2017/25/N/NZ6/00142 (WB).

P.D4.04.03

Antimicrobial peptide derived from chemotactic factor and adipokine -chemerin provides protection against skin invading bacteria by targeting bacteria inner membrane

J. Cichy[1], *U. Godlewska*[1], *A. Zegar*[1], *B. Bilska*[2], *P. Kuleta*[1], *E. Pyza*[2], *A. Osyczka*[1], *B. A. Zabel*[3];
[1]*Faculty of Biochem. Biophys. & Biotech. Jagiellonian Univ., Krakow, Poland,* [2]*Institute of Zoology and Biomedical Reserach, Jagiellonian Univ., Krakow, Poland,* [3]*Palo Alto Veterans Institute for Research, Palo Alto, United States.*

Antimicrobial peptides originating from endogenous human proteins have received significant attention as potential drug targets. Chemerin is chemoattractant and adipokine. Given abundance of chemerin in epidermis and subcutaneous fat tissue, chemerin and chemerin-derived peptides may confer protection against skin invading microbes. Therefore, understanding the modes of action of peptide 4 (p4), the most potent antimicrobial chemerin derivative is of high significance. Here we demonstrate that p4 displayed killing activity against pathogenic MRSA strains *in vitro* and suppressed microbial growth *in vivo* in mouse topical skin and subcutaneous infection models. We also show that p4 binding to bacteria and its bactericidal activity were critically dependent on formation of disulfide-stabilized dimers, suggesting that p4 acts as antimicrobial agent under oxidized conditions. High doses of p4 rapidly damaged internal membrane of bacteria but did not cause lysis of human erythrocytes. P4 in either lethal or sublethal concentration was found to interfere with bacteria respiratory chain function by inhibiting cytochrome bc1-dependent pathway. These data provide new insights into how chemerin shapes host defense by showing previously uncharacterized mechanisms of antimicrobial activity of chemerin derived peptide.
This work was supported by Polish National Science Center grant UMO 2014/12/W/NZ6/00454. The Faculty of Biochemistry, Biophysics and Biotechnology of the Jagiellonian University is a partner of the Leading National Research Center (KNOW) supported by the Polish Ministry of Science and Higher Education.

P.D4.04.04

***In vitro* and *in vivo* posology optimization for an original bacterial immunomodulator**

S. I. Ciulean;
'Cantacuzino' National Institute for Medical-Military Research and Development, Bucharest, Romania.

This study aims to evaluate different oral formulations for an original 13 strain, heat inactivated, bile-lysed, gram positive and negative bacterial immunomodulator formerly used as an injectable product.
THP1 differentiated monocytes were exposed to stimuli derived from the immunomodulator's composition. TLR4 blockage effects were determined on similar cultures.
Female BALB/c mice were administered the immunomodulator in food (1ml/day) or water (10%) for 5 days. Cytokines, NFkB and intracellular reactive oxygen species (ROS) were determined in the spleen, bone marrow and Peyer patches and in THP1 culture supernatants. Animal experiments where approved by the Internal Ethics Committee (CE/101/24.06.2016).
Gram negative bacteria determined higher levels of NFkB and proinflammatory cytokines compared to gram positive. An immunomodulator stimuli equivalent induced greater TNFα and IL8 production, as did sodium deoxycholate compared to bile formulation. TLR4 blockage determined lower TNFα secretion compared to the control and stimuli equivalent. Oral administration did not alter animal health. Compared to the control, both immunomodulator variants down regulated serum MMP9 levels, tended to increase IL10 secretion in Peyer patches and to have a proinflammatory effect in water compared to food administration. Bile-lysed immunomodulator water formulation determined high NFkB levels in Peyer patches. Food administration induced a lower medullar ROS production and inconsequential changes in the cytokine profile of culture supernatants from investigated organs.
Our data suggests that both immunomodulatory formulations have pro-inflammatory effects *in vitro* and anti-inflammatory properties *in vivo*, probably due to oral administration.
This study was supported by PN16390207 project granted by The Ministry of Research.

P.D4.04.05

Th17 cytokine profile in active and latent childhood tuberculosis

M. Druszczyńska[1], *S. Wawrocki*[1], *M. Baranowska*[1], *A. Pankowska*[2], *M. Kowalewska-Pietrzak*[2];
[1]*Division of Cellular Immunology, Department of Immunology and Infectious Biology, Faculty of Biology and Environmental Protection, University of Lodz, Łódź, Poland,* [2]*Regional Center Hospital for Tuberculosis Lung Diseases and Rehabilitation in Lodz, Łódź, Poland.*

Introduction: Tuberculosis (TB) as a consequence of *Mycobacterium tuberculosis* (*M.tb*) infection results from breaking the balance between protective immunity and destructive pathology. Aim: Since the correlates of protective immunity against TB are not known, our study of cytokine profiles of sera and soluble effectors of blood cells responding to *M.tb* antigens was aimed at the identification of immunological signatures of protective immunity and *M.tb* induced pathology in childhood TB. Materials and Methods: In total, 161 BCG-vaccinated HIV-negative pediatric patients were investigated. All children underwent standard clinical and radiological examination including the interferon-gamma release assay (IGRA) testing. On the basis of the results of the current diagnostics children were classified into three groups: TB children, IGRA(+) and IGRA(-) children. A 15-plex Human Th17 Panel (Bio-Rad) was used to measure the concentration of IL-17 pathway-related cytokines in serum and plasma samples recovered from whole blood cultures stimulated with *M.tb* specific antigens performed during IGRA testing. Results: The quantification of 16 proinflammatory and regulatory cytokines and chemokines showed that serum levels of IL-4, IL-10, IL-21 and IL-22 were significantly higher in TB patient group than IGRA(+) children. Moreover, the concentration of IP-10 in *M.tb*-stimulated cultures of blood from TB children was significantly increased as compared to IGRA(+) and IGRA(-) pediatric patients. Conclusion: The analysis of serum/whole blood cultures cytokine profiles may be a useful correlate of active TB in children. This work was supported by the National Science Centre Grant no. 2016/21/B/NZ7/01771.

P.D4.04.06

Il-10 overexpression during the initial steps of infection mediates susceptibility to*Leishmania donovani*infection

I. Mesquita[1,2], ***C. Ferreira***[1,2], *A. M. Barbosa*[1,2], *C. M. Ferreira*[1,2], *D. Moreira*[1,2], *A. Carvalho*[1,2], *C. Cunha*[1,2], *F. Rodrigues*[1,2], *R. Dinis-Oliveira*[3,4,5], *J. Estaquier*[6,7], *A. Castro*[1,2], *E. Torrado*[1,2], *R. Silvestre*[1,2];
[1]*Life and Health Sciences Research Institute (ICVS), Braga, Portugal,* [2]*ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal,* [3]*IINFACTS – Institute of Research and Advanced Training in Health Sciences and Technologies, Department of Sciences, University Institute of Health Sciences (IUCS), CESPU, CRL, Gandra, Portugal,* [4]*UCIBIO, REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal,* [5]*Department of Public Health and Forensic Sciences, and Medical Education, Faculty of Medicine, University of Porto, Porto, Portugal,* [6]*CNRS FR 3636, Université Paris Descartes, Paris, France,* [7]*7Centre de Recherche du CHU de Québec, Université Laval, Québec G1V 4G2, Canada.*

Leishmaniasis is a vector-borne disease caused by protozoan parasites from the genus *Leishmania*. The most severe form of disease is visceral leishmaniasis (VL), which is fatal if left untreated. It has been demonstrated that interleukin (IL)-10, a potent anti-inflammatory cytokine, is associated with disease progression and susceptibility.
We took advantage of a transgenic mouse model that expresses high levels of IL-10 upon zinc sulfate administration (pMT-10). We addressed the role of IL-10 during the initial stages of *L. donovani* infection by analyzing the parasite burden in the spleen and liver of the infected pMT-10 and WT mice as well as the histopathological alterations upon IL-10 induction. Furthermore, the profile of cytokines expressed by T cells was assessed.
Our results demonstrate that high levels of IL-10 during the initial 12 days of infection leads to a higher susceptibility to VL, demonstrated by increased parasite burden in the spleen and the liver. This increased susceptibility of pMT-10 animals was also associated with increased serum levels of alanine transaminase and aspartate transaminase, usually related with hepatic toxicity. Interestingly, the observed phenotype is also correlated with a decreased frequency of multifunctional CD4 T cells and decreased IFN-γ/IL-10 ratio, usually associated with susceptibility against *Leishmania* infection. Such immunologic landscape contributes for the establishment of a successful infection
Our data suggests that overexpression of IL-10 during the initial steps of the infection impacts host ability to control *L. donovani* infection by limiting the development of a protective adaptive immune response.

P.D4.04.07

Human dendritic cell sequestration onto the *Necator americanus* larval sheath during ex-sheathing: a possible mechanism for immune privilege

A. Hassan[1], *A. M. Ghaemmaghami*[1], *D. I. Pritchard*[2];
[1]*Division of Immunology,School of Life Sciences,Faculty of Medicine & Health Sciences, University of Nottingham, Nottingham, United Kingdom,* [2]*School of Pharmacy, University of Nottingham, Nottingham, United Kingdom.*

Despite the profound health implications of Necator americanus infection in humans, many aspects of its interaction with the host immune system are poorly understood. Here we investigated the early events at the interface of N. americanus larvae (L3) and human dendritic cells (DCs). Our data show that co-culturing DCs and the larvae trigger ex-sheathing of hookworms rapidly where a majority of DCs are sequestered onto the larval sheath allowing the ex-sheathed larvae to migrate away unchallenged. Intriguingly, DCs show negligible interaction with the ex-sheathed larvae, alluding to differences between the surface chemistry of the larva and its sheath. Furthermore, blocking of two key C-type lectin receptors on DC surface (i.e. DC-SIGN and mannose receptor) resulted in inhibition of ex-sheathing process and DC sequestration, highlighting the importance of C-type lectins on DCs in the induction of the ex-sheathing. Analyses of DC phenotype and cytokine profile after co-culture with the N. americanus larvae showed an immature phenotype as evidenced by the low expression of the maturation markers and cytokines. These data provide new insights into early events at the interface of human DCs and N. americanus larvae and could explain how L3 evade immune recognition upon initial interaction with DCs.

P.D4.04.08

NEK7 deglutathionylation by GSTO1-1 is required for NLRP3 inflammasome activation

M. M. Hughes*;*
Trinity Biomedical Sciences Institute, Dublin, Ireland.

The NLRP3 inflammasome is a cytosolic complex sensing phagocytosed material and various damage associated molecular patterns, triggering production of the inflammatory cytokines IL-1β and IL-18, promoting pyroptosis. Here, we characterise Glutathione transferase omega 1-1 (GSTO1-1) as a critical NLRP3 inflammasome regulator. Using a small molecule inhibitor of GSTO1-1 termed C1-27, endogenous GSTO1-1 knockdown and GSTO1-1-/- mice, we report that GSTO1-1 is required for NLRP3 inflammasome activation. Mechanistically, GSTO1-1 deglutathionylates cysteine 253 in NIMA related kinase 7 (NEK7) to drive NLRP3 activation. This is the first report of GSTO1-1 as an NLRP3 inflammasome component, and also identify GSTO1-1 as a drug target to limit NLRP3 inflammasome-mediated inflammation.

P.D4.04.09

Identification of the first naturally processed CD4+ T cell epitope of mumps virus

P. Kaaijk*, M. E. Emmelot, M. C. Poelen, W. Han, C. A. van Els, J. de Wit;*
RIVM, Bilthoven, Netherlands.

Several mumps outbreaks have been reported amongst young adults despite vaccination. Poor induction of the T cell response after vaccination may play a role, but has not yet been studied extensively. T cell epitopes can be useful in exploring T cell responses against mumps virus in more detail, but so far no epitope has been identified. Mumps virus nucleoprotein may be a good target for T cell responses as it has been indicated as immunodominant protein, and other viral nucleoproteins have already shown to be a major T cell target. A $CD4^+$ T cell clone was generated from a mumps case using recombinant mumps nucleoprotein as antigen. The T cell clone proved to be directed against a naturally processed epitope, as it recognized mumps virus-infected cells. Using a 2D-matrix peptide pool of 15-mers peptides covering the complete protein, the peptide-specificity could be identified as GTYRLIPNARANLTA. Upon peptide-specific stimulation, the T cell clone responded in a HLA-DR restricted manner by expression of the activation marker CD137, production of IFN-γ, TNF and IL-10, and by exerting different cytotoxic properties. The epitope seems to be widely applicable as it contains binding motifs for at least 12 HLA-DR molecules and is conserved amongst various mumps virus strains. Good (cytotoxic) $CD4^+$ T cell responses against the epitope found in all 8 tested mumps cases indicated its clinical relevance. Therefore, the mumps-specific $CD4^+$ T cell epitope could provide a useful tool to detect and characterize mumps-specific T cell responses following mumps virus infection or vaccination.

P.D4.04.10

Glycan-mediated binding of extracellular vesicles from *Schistosoma mansoni* juvenile worms to DC-SIGN on dendritic cells triggers cytokine release

M. E. Kuipers[1,2], *E. N. Nolte-'t Hoen*[2], *A. J. van der Ham*[1], *A. Ozir-Fazalalikhan*[1], *D. L. Nguyen*[1], *K. F. Hoffmann*[3], *H. H. Smits*[1], *C. H. Hokke*[1];
[1]*LUMC, Leiden, Netherlands,* [2]*Utrecht University, Utrecht, Netherlands,* [3]*Aberystwyth University, Aberystwyth, United Kingdom.*

Extracellular vesicles (EV) are known intercellular communicators and can transport various molecular cargo. Although it is known that the parasitic worm *Schistosoma mansoni* releases EV, their exact composition and interaction with the host immune system are largely uncharacterised. One of the main classes of molecules from schistosomes involved in parasite-host interaction are glycans. Therefore, we investigated the glycosylation of EV released by *S. mansoni* juvenile worm (schistosomula) and their effect on human monocyte-derived dendritic cells (moDC), known to be key cells affected by schistosome-derived products.

Mass spectrometric glycosylation analysis, performed on EV obtained from cultured schistosomula, revealed the composition of N-glycans on the in- and outside of the EV: i.e. expressing oligomannose and Galβ1-4(Fucα1-3)GlcNAc (Lewis^x) motifs, respectively. To investigate binding of EVs by host immune cells, moDCs were incubated with fluorescently labelled vesicles for 1h. Subsequent flow cytometric analysis showed a dose-dependent binding of the EV to moDCs. This binding was significantly reduced after preincubation with anti-DC-SIGN antibodies but not in the presence of anti-mannose receptor or isotype control when measuring the cells after 48h stimulation. In addition, EV induced IL-12 release by moDC was also reduced by blocking DC-SIGN. This suggests that schistosomula EV affect host immune cells via the C-type lectin receptor DC-SIGN. Because DC-SIGN is capable of binding mannose and fucose containing ligands on EV, these results are in agreement with the glycan analysis.

P.D4.04.11

Adenosine-mediated immunomodulatory action on alveolar macrophages during *Klebsiella pneumoniae* B5055 induced acute lung infection

***V. Kumar**, S. Chhibber;*
Panjab University, Chandigarh, India.

Adenosine is considered as a potent metabokine with potential immunoregulatory function. It is produced during condition causing metabolic stress including hypoxia and inflammation. In the current study, we have investigated the effect of 2-cholroadenosine (2-CADO) an analog of adenosine on the lung innate immune response during acute lung infection induced by *Klebsiella pneumoniae* B5055. Acute lung inflammation was induced by intranasal instillation of *K. pneumoniae* B5055 into mice. Subsequently, mice were treated with 2-CADO (10 µg/kg/day/iv) using a treatment schedule. 2-CADO treatment modulated the pro-inflammatory function of alveolar macrophages by significantly ($p \leq 0.05$) decreasing their phagocytic activity, nitric oxide (NO) and hydrogen peroxide (H_2O_2) release. 2-CADO also significantly ($p \leq 0.05$) decreased neutrophil infiltration into the lungs. Levels of pro-inflammatory cytokines (IL-1α and TNF-α) were decreased significantly ($p \leq 0.05$) decreased. However, levels of IL-10 were found to be significantly ($p \leq 0.05$) elevated. Thus, adenosine, a metabokine has promising immunomodulatory action during gram-negative bacterial pneumonia.

P.D4.04.12

Toll-like receptor 7 sustains early VSV replication in draining lymph nodes and prevents VSV-induced neurovirulence

*G. Solmaz[1], F. Puttur[1], M. Francozo[1], **M. Lindenberg**[1], M. Gohmert[1], M. Swallow[1], C. Detje[2], V. Duhan[3], V. Khairnar[3], U. Kalinke[2], B. Ludewig[4], B. E. Clausen[5], H. Wagner[6], K. S. Lang[3], T. Sparwasser[1];*
[1]Institute of Infection Immunology, Twincore, Hanover, Germany, [2]Institute of Experimental Infection Research, Twincore, Hanover, Germany, [3]Institute of Immunology, University of Essen, Essen, Germany, [4]Institute of Immunobiology, Kantonsspital St. Gallen, St. Gallen, Switzerland, [5]Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany, [6]Institute for Medical Microbiology, Immunology and Hygiene, Technical University Munich, Munich, Germany.

Vesicular stomatitis virus (VSV) is an insect-transmitted rhabdovirus that is neurovirulent in mice. Upon peripheral VSV infection, CD169$^+$ subcapsular sinus (SCS) macrophages capture VSV in the lymph, support viral replication and prevent CNS neuroinvasion. Till date, the precise mechanisms controlling VSV infection in SCS macrophages remain incompletely understood. Here we show that toll-like receptor 7 (TLR7), the main sensing receptor for VSV, is central in mechanistically controlling lymph-borne VSV infection. The absence of TLR7 function impedes VSV infection in the draining lymph nodes (dLN), attenuates viral replication and fuels CNS neuroinvasion. By generating novel TLR7 floxed mice, we interrogate the cell-specific importance of TLR7 and demonstrate for the first time that mice lacking TLR7 function in SCS macrophages showed a trend towards reduced VSV titers in the dLN, whereas CD11c- and LysM-Cre conditional TLR7 knock-out mice showed no reduction. Overall, we identify TLR7 as an essential host factor that facilitates VSV replication, promotes anti-VSV immunity and controls the onset of neurovirulence.

P.D4.04.13

Treatment with recombinant interleukin-12 protects against secondary cystic echinococcosis

***D. MEZIOUG**, C. Touil;*
Laboratory of Cellular and Molecular Biology- Faculty of Biological Sciences- University of Sciences, Algiers, Algeria.

Human cystic echinococcosis is a parasitic disease caused by the development in humans and other mammals by the larval form of Taenia *Echinococcus granulosus*. The present studies aimed to identify anti hydatic molecules, which reduce the risk of relapse during hydatidosis. We investigate *in vitro,* IFN-γ, IL-12, IL-4, IL-13, IL-10 and TGF-β effect on the protoscoleces of *Echinococcus granulosus* in co-cultures with peripheral blood mononuclear cell (PBMC) and monocytes/macrophages (Mo/Mac) from hydatid patients (n=65). We observed 20 relapse cases. The supernatant was collected for nitrite (NO_2- +NO_3) and urea determination. PSC viability was assayed using eosin staining. Our results showed that NO levels were significantly higher in supernatants of co-cultures treated with IFN-γ, IL-12 when compared to untreated cultures supernatants ($p<0.01$). This production was concomitant with a decrease in the percentage of viable protoscoleces. However, the treatment of co-cultures with exogenous IL-4, TGF-β down regulated the NO production and enhanced protoscolices viability and urea production. We observed with interest relapsing patients did not respond to protoscoleces stimulation and their PBMC and Mo/Mac did not secrete significant amounts of NO when stimulated with parasitic antigen. The reduction of nitrite production in relapsing patients correlates with the lack of IFN-γ and decrease in production of IL-12. Interestingly, addition of IL-12 to co-cultures of relapsing patients reduced protoscoleces viability. Collectively, our results show that IL-12 cytokines plays a relevant role in the scolocidal activity of mononuclear cells. Our finding may provide an alternative approach to the treatment of patients with hydatic disease.

P.D4.04.14

Neutrophil surface phenotype and oxidative output alterations in sepsis

***I. Obraztsov**[1], A. Ryabov[2], M. Sukhina[1], N. Tsuranova[1], E. Balykova[1], A. Paramonov[1];*
[1]A.N. Ryzhikh State Scientific Centre for Coloproctology, Moscow, Russian Federation, [2]Ludwig-Maximilians-Universität, München, Germany.

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Neutrophils play a key role in initializing and regulating host response, neutrophil functional deficiency might lead to sepsis development and progression.
Our aim was to evaluate the kinetics of the neutrophil's oxidative output and surface phenotype features associated with surgical sepsis development.
Oxidative metabolism was evaluated in the blood by means of luminol-enhaced chemiluminescence (CL) induced by incubation with 50 ng/ml 4-phorbol-12-myristate-13-acetate (PMA) prior to 10 µM N-formyl-methionyl-leucyl-phenylalanine (fMLP) stimulation. This approach enabled to obtain a signal of high intensity and reproducible pattern. Phenotypic shift kinetics of the neutrophils under our assay conditions was also investigated by means of flow cytometry; we revealed the time-dependent change of CD62L, CD11b and CD15 after PMA stimulation. Assay was held in a control group of 95 healthy individuals to build a reference for CL parameters. Investigation of 17 patients with severe burns in dynamics revealed that insufficiency of intracellular CL was associated with development of severe septic complications. A linear combination of 3 fitting power exponent functions (1) was suggested to model experimental CL curves and clinically relevant kinetic parameters were substantiated.
y(t)=A*(t-t0)^n*e(- (t-t0)/τ) (1)
Investigation of 86 colorectal cancer patients after surgery revealed that increase of sequential organ failure assessment score is associated with the increase of CD64 and CD44 expression ($p < 0.05$) as well as the increase and subsequent loss of intracellular CL intensity. The proposed parameters are promising for early sepsis diagnosis.

P.D4.04.15

Therapeutic effect of DNA vaccine containing the 60-kDa-heat shock protein gene from *Paracoccidioides brasiliensis* in experimental paracoccidioidomycosis in mice

*I. L. Souza[1], F. F. Fernandes[1], M. L. Schiavoni[1], **A. Panunto-Castelo**[2];*
[1]Faculdade de Medicina de Ribeirão Preto - Universidade de São Paulo, Ribeirão Preto, Brazil, [2]Departamento de Biologia - Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto - Universidade de São Paulo, Ribeirão Preto, Brazil.

Paracoccidioides brasiliensis and *P. lutzii* are the etiological agents of paracoccidioidomycosis (PCM). PCM is the most prevalent deep mycosis in Latin America and Brazil, which holds most of the cases (~80%). We have previously shown that *P. brasiliensis*-infected mice treated with 50 µg of recombinant 60-kDa-heat shock protein from *P. brasiliensis* (rPbHsp60) had a worsening of infection. Here we analyzed whether the treatment of infected mice with PbHsp60 gene cloned into a plasmid (pVAX1-*PB_HSP60*) would render an efficient immune response and improvement of the disease. To this end, mice were infected intratracheally with 10^5 *P. brasiliensis* yeasts and, on day 20 postinfection, treated intramuscularly with either only one dose or three doses at 15-days interval of 100 µg pVAX1-*PB_HSP60* or empty plasmid, 50 µg rPbHsp60 or vehicle. Thirty days after treatments, lungs from mice that received only one dose of rPbHsp60 had 3-fold higher fungal burden, a more severe inflammatory reaction and a higher concentration of cytokines IL-6, IL-10, and IL-17 when compared with the other groups. Treatment with one dose of pVAX1-*PB_HSP60* did not show a considerable difference compared with controls. In contrast, infected mice treated with three doses of pVAX1-*PB_HSP60* or rPbHsp60 had a significant decrease in the fungal burden, milder inflammation, a higher concentration of cytokines IFN-γ, TNF-α, IL-6, and IL-17 and a lower amount of IL-10 when compared with controls. The detrimental impact of a single-dose treatment with rPbHsp60 on experimental PCM in mice is under investigation. Financial support: FAPESP (2017/01390-8).

P.D4.04.16

Using oligonucleotides to control a dysregulated type I interferon response in severe influenza

***J. C. F. Rappe**, A. Wack;*
Francis Crick Institute, London, United Kingdom.

Influenza virus infection causes respiratory disease leading to 500.000 deaths each year worldwide. During infection, one important component of the innate immune response is the expression of type I interferons (IFN-I). Signalling through its receptor IFNAR1/IFNAR2, this cytokine family will interfere with viral replication and induce an antiviral state in infected and bystander cells. Many publications demonstrated the beneficial effects of a moderate IFN-I response during infection. However, in humans, severe influenza is associated with high IFN-I levels. Similarly, mouse strain 129 responds to influenza infection by producing excessive IFN-I amounts that drive pathogenic innate immune responses. Genetic removal of IFN-I signalling ameliorates disease. Depletion experiments determined that IFNα was mainly produced by plasmacytoid dendritic cells (pDCs). The signal inducing pDCs to produce IFNα in influenza infection is commonly thought to be Toll-like receptor 7 (TLR7)-mediated recognition of viral RNAs. While Ab-mediated pDC depletion ameliorated disease in severe influenza in 129 mice, a pharmacological approach using small molecules would be preferable and of clinical interest. Thus, the oligonucleotide antagonist of TLR7 661 was tested in vitro and showed a high efficacy at reducing influenza-triggered IFNα production by bone marrow-derived pDC already at 0.75uM. In vivo, 661 anti-IFNα effects during influenza infection were observed only when administered locally (intranasally) at a dose of 4.8mg/kg. Studies are currently ongoing to determine if reducing excessive IFNα production during influenza infection in the pro-inflammatory 129 model will reduce overall influenza severity.

P.D4.04.17

***Candida albicans* CRASP11 modulates the host-mediated dendritic cell response by binding the pattern-recognition-receptors dectin-1 and dectin-2**

***N. Reiher**[1,2], M. Reza[2], C. Skerka[2], P. Zipfel[2,1,3];*
[1]Center for Sepsis Control and Care, Jena, Germany, [2]HKI-Leipniz Institute for Natural Product Research and Infection Biology, Jena, Germany, [3]Freidrich-Schiller University, Jena, Germany.

Introduction: The human organism is continuously challenged by an arsenal of microorganisms. To detect and clear these microbes and to maintain homeostasis it is fundamental to recognize these intruders. Therefore, the host utilizes dendritic cells (DC) that express pattern-recognition-receptors (PRR) which bind highly conserved microbial-associated-molecular-patterns (MAMPS). Dectin-1 and dectin-2 are mainly responsible for detection of fungal pathogens. *Candida albicans* an obligate pathogenic fungus colonizes mucosal surfaces in most humans without causing symptoms. However, in immunosuppressed patients candida can cause superficial and severe systemic infections that have lethal outcome. Aim of this project is to characterize how candida by secreting immune modulatory proteins interferes with immune recognition.
Methods: Candida protein CRASP11 is expressed in *E. coli*. For binding analyzes Biolayer Interferometry, Confocal Microscopy, ELISA and Western Blot were used. For functional studies dendritic cells were generated and function was assayed with cytokine measurements and Flow Cytometry.
Results: We identified *Candida albicans* CRASP11 as a new ligand for both dectin-1 and dectin-2. CRASP11 is surface exposed and secreted and binds dectin-1 and dectin-2 on the surface of monocyte-derived DC. The CRASP11::dectin-complexes have affinities of 10 and 200 nM. By binding dectin-1 and dectin-2 CRASP11 activates DC and induces expression of maturation marker CD83 and CD86 and the production of pro- and anti-inflammatory cytokines.
Conclusion: *Candida albicans* CRASP11 can bind both dectin-1 and dectin-2 with high affinities and by binding to the surface of dendritic cells it induces cell activation and cytokine response.

P.D4.04.18

Influenza virus infection induces *Heatr9* upregulation in lung alveolar epithelial cells

***C. J. Stairiker**[1,2], M. v. Meurs[1], Y. Mueller[1], I. Brouwers-Haspels[1], S. Erkeland[1], P. Katsikis[1];*
[1]Erasmus University Medical Center, Rotterdam, Netherlands, [2]Drexel University College of Medicine, Philadelphia, United States.

Influenza virus infection poses a serious threat to public health. It infects lung alveolar epithelial cells in the respiratory tract, utilizing them to reproduce, spreading the infection. Understanding genes that are dynamically regulated during influenza virus infection may help elucidate essential genes and pathways that affect influenza virus infection. We examined the *in vivo* RNASeq gene expression profiles of alveolar epithelial cells sorted from A/Puerto Rico/8/1934-GFP (PR8-GFP) expressing influenza virus infected mice. We identified a novel influenza virus-induced gene, *Heatr9* (also known as *Gm11435*), that was upregulated by >200-fold in vivo in mouse alveolar epithelial cells. The upregulation of *Heatr9* by influenza virus was further confirmed *in vitro* in PR8-GFP influenza virus infected human lung A549 cells. *In vivo Heatr9* upregulation was found to be indirect as bystander alveolar epithelial cells in lungs exhibited similar levels of *Heatr9* induction as infected cells. Furthermore, supernatants of influenza virus infected A549 cells were capable of potently inducing *Heatr9* mRNA even in the absence of infection. To identify factors that upregulate *Heatr9* we examined the effect of cytokines on *Heatr9* expression in vitro. Although not induced by IFNβ, TNFα, and IL-1β alone in A549 cell, when used in combination, IFNβ and TNFα or IFNβ and IL-1β potently induced *Heatr9* mRNA. Currently, we are generating Heatr9 deficient cell lines to examine the function of this gene in infection. In summary, we have identified *Heatr9* as a cytokine- and influenza virus infection inducible gene, the function of which has a yet to be determined.

P.D4.05 Exploiting host pathogen interaction - Part 5

P.D4.05.01

Manipulation of the Th2/1 to Th2 ratio affects the fitness of parasitic nematodes

***N. Affinass**[1], M. Löhning[2], S. Hartmann[1], S. Rausch[1];*
[1]Freie Universität Berlin, Berlin, Germany, [2]DRFZ, Berlin, Germany.

Helminths are highly prevalent in livestock and infect a quarter of the human population. The dominant immune response to helminths is characterized by type 2 T helper (Th2) cells mediating partial immunity to these parasites in the majority of cases. There are no vaccines available for the use in humans. Hence, a better understanding of the immune response to helminth infections is needed.
Heligmosomoides polygyrus is a natural mouse parasite serving as a model for gastrointestinal nematodes chronically infecting humans. We have previously shown that a substantial proportion of helminth-reactive CD4+ T cells display a Th2/1 phenotype by co-expressing the transcription factors GATA-3 and T-bet as well as Th2 cytokines and IFN-γ. To survey if the oral route of infection and the microbiota promote the instruction of Th2/1 cells, the responses to subcutaneous immunization with parasite products and immune responses of germfree mice were compared to those of conventional mice infected with *H. polygyrus.* Th2 and Th2/1 differentiation was similar in all conditions, arguing against a dependency of Th2/1 instruction on microbial signals. However, Th2/1 differentiation was more prominent in more susceptible C57BL/6 compared to more resistant BALB/c mice. In addition, mice lacking the IFN-γ receptor displayed a more pronounced conventional Th2 response which was associated with decreased worm fitness. In conclusion, Th2/1 hybrid cells are a prominent population within the helminth-reactive CD4+ T effector pool. Whether different proportions of conventional Th2 and Th2/1 cells determine the effectiveness of immune responses to nematode infections is the focus of ongoing work.

P.D4.05.02

Systemic bacterial infections affect DCs by favouring monocyte development

*K. Bieber[1], M. Günter[2], **S. E. Autenrieth**[1];*
[1]Department of Internal Medicine II, University of Tübingen, Tübingen, Germany, [2]Departement of Internal Medicine II, University of Tübingen, Tübingen, Germany.

Dendritic cells (DCs) are critical in host defense against infection bridging the innate and adaptive immune system. Animal and human data suggest that systemic DC depletion is an early event in the course of sepsis that may impair the host defense mechanisms. Previously, we showed that systemic bacterial infection irrespectively of the particular pathogen (Gram-negatives such as *E. coli* and *Y. enterocolitica*, Gram-positives such as *S. aureus* and *L. monocytogenes*) reducedthe numbers of bone marrow hematopoietic progenitors of the DC lineages. Moreover, *Y. enterocolitica* infection led to TLR4/IFN-γ-dependent monopoiesis at the expense of DC differentiation. Herein, we extend these studies to gain insights into the mechanisms of DC progenitor reduction as well as impaired *de novo*DC-development upon infections with different Gram-positive and Gram-negative pathogens. The reduction of the DC progenitors was TLR4 dependent for Gram-negative and TLR2-dependent for Gram-positive bacteria. However, induction of monopoiesis by the various infectious agents was TLR-independent. Although the de novo DC generation was dramatically impaired, IFN-γ was dispensable for the induction of DC-progenitor reduction and concomitant monopoiesis upon infections caused by Gram-positive bacteria, indicating the involvement several mechanisms. Our study supports the notion, that systemic bacterial infection leads to general attrition of myeloid progenitors in the bone marrow and DCs in the periphery, which can be compensated by emergency monopoiesis not only to sustain but even to increase the numbers of innate immune monocytes to promote pathogen control.

P.D4.05.03

Characterisation of interferon-induced protein 44 mediated inhibition of respiratory syncytial virus (RSV) infection

D. C. Busse[1], *S. Smith*[2], *C. Brandt*[2], *S. Clare*[2], *P. Kellam*[2], *J. S. Tregoning*[1];
[1]*Imperial College London, London, United Kingdom,* [2]*Wellcome Trust Sanger Institute, Hinxton, United Kingdom.*

RSV infection represents a major global cause of morbidity and mortality, yet the factors that influence disease severity are not fully understood. One possibility is that disease severity is linked to the ability of the infected host cells to recognise and control viral infection. Respiratory viral infection triggers the production of interferons which in turn induce an array of intracellular antiviral proteins encoded by Interferon-stimulated genes (ISGs). Whilst some ISGs have been linked to the development of severe RSV disease, little is known about the mechanism of many of these important antiviral genes. The ISGs IFN-induced protein 44 (IFI44) and IFN-induced protein 44-like (IFI44L) are upregulated after RSV infection, but they do not as yet have a defined mechanism of action. We hypothesised that their upregulation after infection was linked to a protective role. The effects of IFI44 and IFI44L on viral infection were analysed via siRNA-mediated knockdown or through overexpression in relevant human cell lines. The impact of IFI44 on RSV infection was further investigated in *Ifi44*$^{-/-}$ mice. Both IFI44 and IFI44L were confirmed as ISGs upregulated during RSV infection both *in vitro* and *in vivo*. Knockdown of IFI44 in epithelial cell lines resulted in elevated viral infection. RSV infection in *Ifi44*$^{-/-}$ mice was associated with more severe disease relative to wild type controls with increased viral load during infection. This therefore suggests that IFI44 plays an important antiviral role in the prevention of RSV infection.

P.D4.05.04

Beta-glucan modulates human macrophage differentiation and polarization toward macrophages with unique properties

G. Camilli*, J. Quintin;*
Pasteur Institute, Paris, France.

β-glucan, a naturally derived polysaccharide present in the cell wall of fungi, positively impact the outcome of cancer and a number of infectious diseases, although the exact mechanisms remain to be elucidated. Macrophages derived from monocyte precursors undergo specific differentiation that depends on microenvironmental signals. In tissue, they mature and acquire distinct functional phenotype in response to environmental cues. Evidences suggest that β-glucan treatment converts the immunosuppressive M2 (alternatively activated macrophages) and TAM (tumor associated macrophages) toward the M1 (classically activated macrophages) pro-inflammatory and antitumor phenotype. Recently, several seminal studies showed that stimulation of human monocytes by β-glucan bring monocytes into a long-term enhanced functional state, through metabolic and epigenetic changes (trained immunity). Trained monocytes show a stronger proinflammatory response to a second stimulus and provide non-specific protection against several types of infections. Although a stronger proinflammatory response is generally acknowledged as beneficial in controlling infection and cancer progression, it can in some circumstances cause damage to healthy tissue and contribute to pathology. As such, exacerbated secretion of inflammasome-derived cytokines (i.e. IL-1β) have a critical pathogenic role in several inflammatory diseases.
Providing the new concept of trained immunity and its non-specific protective properties, we sought to investigate how the fungal β-glucan modulates the differentiation of human monocytes into macrophages. Here, we show that treatment of human monocytes with fungal β-glucan skews their differentiation, triggered by either M1 or M2 stimuli, into macrophages with a specialized functional phenotype. As such, these macrophages exhibit a non-deleterious secretion of inflammasome-related molecule IL-1β.

P.D4.05.05

Role of Immunoglobulin A in *Mycoplasma pneumoniae* upper respiratory tract carriage

R. C. A. de Groot[1], *P. M. Meyer Sauteur*[2], *L. M. Verhagen*[3], *A. Perkasa*[1], *E. B. Spuesens*[4], *S. E. Estevão*[1], *T. Hoogenboezem*[1], *A. M. van Rossum*[1], *W. W. Unger*[1];
[1]*Erasmus MC, Rotterdam, Netherlands,* [2]*University Children's Hospital of Zurich, Zurich, Switzerland,* [3]*Utrecht University Medical Center – Wilhelmina Children's Hospital, Utrecht, Netherlands,* [4]*Van Weel-Bethesda Hospital, Dirksland, Netherlands.*

Introduction: *Mycoplasma pneumoniae (Mp)* is the most common bacterial cause of community-acquired pneumonia in children. Infection in the lower respiratory tract (LRT) is preceded by asymptomatic carriage in the upper respiratory tract (URT). We studied the role of the humoral response to *Mp* in the URT and compared it to the LRT.
Methods: *Mp* or medium was installed intranasally in C57BL/6 or B cell-deficient μMT mice. Healthy children and children with selective IgA deficiency (sIgAD) were recruited. On respiratory tract samples we determined *Mp* copy number by qPCR and *Mp*-specific IgM, IgA and IgG titers using an in-house ELISA.
Results: In *Mp* infected mice, *Mp*-specific IgG was markedly elevated in the bronchoalveolar lavage fluid. In contrast, the nasal lavage fluid contained high levels of *Mp*-specific IgA. Serum transfer of infected wild-type mice to μMT mice rescued LRT clearance of *Mp.* Interestingly, the serum transfer had no effect on *Mp* load and *Mp*-specific IgA levels in URT. Immunofluorescence showed the presence of IgA-positive B cells in the URT starting from day 7. To translate our findings to children we measured *Mp*-specific antibodies in children. We included 33 children with sIgAD and 477 healthy control subjects.
Conclusions: *Mp*-specific IgG responses dominated in the LRT, whereas *Mp*-specific IgA was increased in the URT, where it seemed to lower *Mp* load. Insights into the humoral response to *Mp* can benefit vaccine development and immunoglobulin treatment of patients with primary antibody deficiency.
Acknowledgement: This research was supported by Sophia Research Foundation (grant S18-04 to RdG)

P.D4.05.06

The effect of immunonutrien intake on severity of pulmonary tuberculosis clinical symptoms

T. Faadhila*, D. Sulastri, A. Ana, I. M. Sari;*
Andalas University, Padang, Indonesia.

Introdution: Tuberculosis (TB) is one of the top 10 causes of death worldwide. The severity of pulmonary TB symptoms is affected by the immune status which is strongly influenced by nutrition. Immunonutrient can be defined as the effect of the provision of specific nutrients on immune function. Vitamin D is known to enhance immune response to mycobacteria. In addition, protein products cytokine which help body defense to mycobacteria. The aim of this study was to determine the effect vitamin D and protein intake as the immunonutrient on severity of pulmonary tuberculosis clinical symptoms.
Materials and methods : The method of this study is cross sectional study of 52 pulmonary tuberculosis patients. They were interviewed with questionnaire of FFQ. The amount of vitamin D and protein intake is calculated by using the nutrisurvey. Clinical symptoms of TB patients are measured by using Bandim TBScore. Statistical analysis of the two variables is allegedly linked using SPSS.
Results : The average intake of vitamin D is 5.86 mcg/day, SD 5.45 . The average intake of protein is 78,477 gram/day, SD 29,30. The respondents who have degree TBScore I is 92.3% (48 persons), TBScore II is 7.69% (4 persons), TBScore III is 0% (0 persons). The average of TBScore is 2,04 ± 1,89.
Conclusions : Based on the statistical analysis, vitamin D and protein intake are not significantly affect the severity of pulmonary TB clinical symptoms. However, the average intake of TBScore II group is much lower than the intake of TBScore I group.

P.D4.05.07

Immunomodulatory role of radiations in the control of Epstein-Barr virus fate

D. Faicchia[1], *C. Procaccini*[1], *S. Bruzzaniti*[1], *C. Fusco*[2], *T. Micillo*[3], *M. Cirone*[4], *A. Faggioni*[4], *G. Matarese*[2];
[1]*Isitituto di Endocrinologia e Oncologia Sperimentale, Consiglio Nazionale delle Ricerche, Napoli, Italy,* [2]*Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università di Napoli "Federico II", Napoli, Italy,* [3]*Dipartimento di Biologia, Università di Napoli "Federico II", Napoli, Italy,* [4]*Department of Experimental Medicine, "Sapienza" University of Rome, Roma, Italy.*

Autophagy is a catabolic pathway involved in cell survival under stress conditions. Cells engage autophagy as a detoxification mechanism and/or to solve infections, but some micro-organisms are learning to evade it or to appropriate of this machinery for their own benefit. Recent studies have highlighted a central role of autophagy in supporting the lytic phase of Epstein-Barr virus (EBV). Furthermore, data from literature have suggested a potential immunomodulatory role exerted by ionizing radiation in controlling viral fate. In this study, we investigated the effect of radiations on immortalized B lymphocytes, which have integrated EBV in episomal form. Upon radiation exposure, EBV-infected B cells showed a reduced activation of the mammalian-target of rapamycin (mTOR) pathway, which resulted in an increased autophagic flux. Concomitantly, in EBV-infected B cells, irradiation induced the expression of proteins of the early phases of EBV lytic cycle (Zebra, EA); these phenomena were accompanied by an enhanced release of pro-inflammatory cytokines (IFN-γ, IL-17, TNF-α), a reduced secretion of anti-inflammatory cytokines (such as IL-10), together with an increased expression of the activation marker CD40 on infected B cells. Taken together, these data suggest a key role of radiation in the loss of EBV latency in B lymphocytes and in the reactivation of viral lytic cycle, through autophagy induction. A better understanding of the mechanisms that regulate the interplay between radiation and viral activation could help to improve the treatment of EBV-associated diseases.

P.D4.05.08

***Listeria monocytogenes* adapt to the host cells by inducing the AP-1/Fra-1 level thus inhibiting the *guanylate-binding proteins* expression**

N. Hannemann[1], *S. Cao*[1], *G. Schett*[1], *D. Soulat*[2], *A. Bozec*[1];
[1]*Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Department of Internal Medicine 3 – Rheumatology and Immunology, Universitätsklinikum Erlangen, Erlangen, Germany,* [2]*Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Institute of Microbiology Clinical Microbiology, Immunology and Hygiene, Universitätsklinikum Erlangen, Erlangen, Germany.*

Macrophages have a broad arsenal of microbicidal features. During *Listeria monocytoges* (*L.m.*) infection, they are the first line of defence against liver and spleen damage. The activator protein (AP)-1 transcription factor family, specifically its subfamily of FOS proteins (cFos, FosB, Fra-1 and Fra-2), can regulate macrophage cytokines production. To delineate whether Fra-1 is involved in the antimicrobial defence of macrophages, chromatin-immunoprecipitation (ChIP) sequencing analysis using thioglycollate-elicited macrophages pooled down for Fra-1 were performed. This analysis revealed that Fra-1 binds to the promoter regions of guanylate-binding protein (Gpb) -11, -2b, 3 and 5. To investigate whether Fra-1 is involved in the defence against *L.m*. infection, bone marrow-derived macrophages (BMDM) from Fra-1 deficient and wildtype mice were infected with *L.m.* (MOI10). Interestingly, we found a decreased CFU in Fra-1 deficient BMDM. In accordance decreased LDH levels reflecting a decreased toxicity was quantified in the supernatant of the Fra-1 deficient cells when compared to controls. RNA analysis of infected BMDM revealed no difference in type-1 interferons, but increased expression of *Gpb-5*, *-6* and *-10*. To address the role of Fra-1 *in vivo*, Fra-1 deficient mice (Fra-1$^{fl/fl}$ MxCre$^{-/+}$) and their wildtype littermates were infected intraperitoneally with *L.m.*. In line with the previous finding, CFU of *L.m.* was reduced in the spleen and liver of Fra-1 deficient mice. Moreover, the expression of *Gbps-5, -6, -7* and *-10* was increased in the liver and spleen of Fra-1 deficient mice. Our data suggest that Fra-1 in macrophages inhibits microbicidal factors, especially GBPs, promoting bacterial growth.

P.D4.05.09

Sirtuin 5 deficiency does not compromise innate immune responses to bacterial infections

T. Heinonen[1], *E. Ciarlo*[1], *C. Théroude*[1], *A. Pelekanou*[1], *J. Herderschee*[1], *D. Le Roy*[1], *J. Auwerx*[2], *T. Roger*[1];
[1]*Lausanne University Hospital, Epalinges, Switzerland,* [2]*Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.*

Background and aim: Sirtuins (SIRT1-7) belong to the highly conserved family of NAD$^+$-dependent lysine deacetylases. SIRT5, one of the least characterized sirtuins, resides mainly in the mitochondria and catalyses lysine deacetylation, demalonylation, desuccinylation and deglutarylation to regulate metabolic and oxidative stress response pathways. Pharmacologic inhibitors of SIRT5 are under development for oncologic conditions. Nothing is known about the role of SIRT5 in innate immune responses. The aim of the study was to investigate whether SIRT5 deficiency impacts on host defenses against infection.
Methods: Mice were housed in SPF conditions. Thymic and splenic subpopulations were analyzed by flow cytometry. Bone marrow-derived macrophages and splenocytes were stimulated with TLR ligands, bacteria, exotoxins and polyclonal activators and analyzed for metabolic status, cytokine production and proliferation. Mice were challenged i.p. with LPS or *E.coli*, i.n. with *K.pneumoniae* or *S.pneumoniae* and i.v. with *L.monocytogenes* or *S.aureus*. Blood was collected to quantify cytokines and bacteria. Weight, severity score and survival were registered.
Results: SIRT5 deficiency did not affect immune cell development. SIRT5 deficiency increased oxidative phosphorylation over glycolysis in macrophages, but did not modulate cytokine production and proliferation by macrophages and splenocytes. SIRT5 deficiency had no impact on cytokine blood levels, bacteremia and survival rates in models of endotoxemia, pneumonia, peritonitis, listeriosis and staphylococcal sepsis.
Conclusions: These data suggest that SIRT5 has no major impact on innate immune responses to bacterial infections and support the safety profile, in terms of susceptibility to infections, of SIRT5-directed therapies under development for oncologic diseases.

P.D4.05.10

Platelets and the regulation of tissue destruction in tuberculosis.

D. E. Kirwan[1], *A. M. Whittington*[1], *K. A. Fox*[1], *R. H. Gilman*[2], *K. A. Taylor*[1], *M. Emerson*[1], *J. S. Friedland*[1];
[1]*Imperial College London, London, United Kingdom,* [2]*Johns Hopkins University, Baltimore, United States.*

Introduction Tuberculosis (TB) is characterised by inflammation and immune-mediated tissue damage by enzymes, particularly matrix metalloproteinases (MMPs). Platelets are increasingly recognised as immune regulators but their role in TB is poorly understood.
Methods Platelet-derived markers were measured in 50 TB patients before and during treatment, and in 50 healthy controls.
Fresh platelets were incubated with live, virulent TB, TB-derived secreted antigens, or control medium for 30min. Monocytes were cultured with autologous platelets ±TB for 24h. Supernatants were analysed by ELISA and gene expression by qPCR. Platelet function was assessed using light transmission aggregometry.
Results Platelet factor 4 (PF4) concentrations were significantly higher in TB patients than healthy controls (median 1,129 [IQR 1,769] vs 462.5 [IQR 693] ng/ml, p<0.0001). PF4 concentrations transiently increased at treatment day 14 and normalised by day 60 (612.7 [IQR 1,806] ng/ml, p=0.073 vs controls). CD40L, PDGF-BB, and PTX-3 concentrations followed similar trends.
Incubation with TB did not affect platelet PF4 secretion but soluble TB antigens increased PF4 secretion from 141.8 ±6.6 to 255.8 ±13.8 ng/ml (p=0.0017) and also impacted functional platelet responses. Platelets significantly increased MMP-1 and -10 secretion from TB-infected monocytes from 231.9 ±29.1 to 1,820 ±59.2 pg/ml (p=0.0017) and from 530.8 ±45.3 to 1,341 ± 84.2 pg/ml (p=0.014) respectively. MMP-1 and -10 gene expression were similarly upregulated.
Conclusion Platelet activity is increased in TB patients, which is driven by TB-secreted antigens. Platelets drive tissue-degrading enzyme secretion by TB-infected monocytes which may contribute to TB immunopathology. Platelets may constitute a potential therapeutic target for TB.

P.D4.05.11

Effector mechanisms of neutrophilic granulocytes in realization of systemic and local immune response in children suffering from purulent-inflammatory diseases of soft tissues

I. V. Nesterova[1], *G. A. Chudilva*[2], *V. A. Tarakanov*[2], *N. K. Barova*[2], *T. B. Rusinova*[2], *S. V. Kovaleva*[2], *A. A. Evglevsky*[2];
[1]*Peoples' Friendship University of Russia, Moscow, Russian Federation,* [2]*Kuban State Medical University, Krasnodar, Russian Federation.*

Neutrophilic granulocytes (NG) play a crucial role in antibacterial defense realizing its functions by phagocytose, transmembrane degranulation, formation of neutrophilic extracellular traps (NET) and ectosomes. Staphylococcus(Stph.), causing the purulent-inflammatory diseases of soft tissues, emit virulence factors that violate the mechanisms of NG' phagocytosis. We had studied 16 children (group 1), 3-8 years, suffering from purulent-inflammatory diseases of soft tissues (abscesses, phlegmons). Control group consist 7 healthy children. In both groups we evaluated phagocytosis NG of peripheral blood (PB), using Stph.aureus 209: phagocytic activity - % PAN, capture (PN, IP), digestion (% D, DI) functions. NG ability to form NET was tested in smear-prints from site of purulent inflammation (PI) in group 1. NG PB of patients of group 1 had shown inadequate response on the purulent process, causing by Stph.aureus or Stph.epidermidis: NG number and their phagocytic activity didn't differ significantly from control. In smear-prints % PAN was in 1,4 times lower than in PB - 37,5[20;39]%, PN, IP was reduced. Formation of NET was visualized in smear-prints. The number of NET was 21.3% [14,7:31,95] relatively to NG total number. The realization of NG bactericidal potential in site of PI is carried out by 2 mechanisms - phagocytosis and NET formation. We had concluded, that ineffective phagocytic activity of PB NG in systemic immune response, defects of phagocytosis of NG in the local site of PI were associated with the damaging influences of Stph. We assume that the formation NET compensated impaired phagocytic function of NG in site of PI.

P.D4.05.12

Mitocondrial dynamic, beta tubulin and extracellular traps in cultures of human autologous leukocytes stimulated with LPS

R. Rinero, M. V. Reyna, F. M. Rodriguez, ***I. Novak****;*
Institute of Cell Biology, Faculty of Medicine, Cordoba, Argentina.

Introduction: the endotoxemia produced by the effects of endotoxins such as LPS in the blood circulation lead to inflammation in multiple organs. Extracellular traps (ETs) are structures of chromatin and intracellular proteins which are extruded in leukocytes in inflammatory conditions. Some protein components of the cytoskeleton have been described in the traps, but the presence of beta-tubulin has not been reported. Not all Ets are created equal, this depend on source of stimulation. Mitochondria are currently considered to have regulatory functions of innate and adaptive immunity and are determinants in the phenotypes adopted by immune cells in their responses. Objectives: generation of ETs in leukocyte cultures, challenged with LPS and perform marking beta-tubulin and on the other hand, to observe the morphology characteristics of mitochondria of lymphocytes in LPS assay. Methods: autologous leukocyte cultures from healthy human blood samples with ethical consent, anticoagulated with heparin were stimulated with 25 ng/ml LPS, 30 min. Immunofluorescence technique with anti-beta-tubulin Abs, DNA stain with DAPI. Paired blood samples provided controls. Cell pellets from cultures were performed to studied with electron microscope transmission. Results: beta-tubulin molecules were observed in ETs. We observed altered mitochondrial morphology in samples of LPS assay with an increase in size and cristae complexity with electrolucid images in lymphocytes. Conclusions: the expression of beta-tubulin allow to better understand the composition of ETs generated by LPS. Is this similar in the ETs triggered by different stimulous? Mitochondrial morphological changes conduce to improve or deteriorate lymphocyte functions? Further experiments are necesary.

P.D4.05.13

CD72/CD100 and PD-1/PD-L1 markers are increased on T and B cells in HIV-1 viremic patients, and CD72/CD100 axis is correlated with T-cell exhaustion

R. Correa-Rocha, J. Lopez-Abente, V. Perez-Fernandez, A. Prieto-Sanchez, M. Muñoz-Fernandez, ***M. Pion****;*
Instituto de Investigacion Sanitaria Gregorio Marañon, Madrid, Spain.

During HIV-1 infection, PD-1/PD-L1 axis' role in dysfunction of the immune response was described, and high expression of PD-1 and PD-L1 was associated with an immunosuppression state by limiting HIV-1-specific T-cell response. On the other hand, CD100 was demonstrated to play a relevant role in immune response and its expression at the surface of T cells is unknown, although it may play a role in immune deregulation during HIV-1 infection. We researched the PD-1/PDL-1, and CD72/CD100 axes-related markers expression on T and B cells in HIV-1$^+$ naïve-treated patients and in healthy individuals. We analyzed the frequencies and fluorescence intensities of these four markers on CD4$^+$ and CD8$^+$ T cells and on B cells. Expression of these markers was increased during active HIV-1 infection. The frequency of CD100 on T cells was positively associated with the expression of PD-1 and PD-L1 on T cells from naïve-treated HIV-1$^+$ patients. In addition, the frequency of CD72-expressing T cells was associated to the IFN-γ production in naïve-treated HIV$^+$ patients. Our data suggest that CD72/CD100 and PD-1/PD-L1 axes all together may participate in deregulation of immunity during HIV-1 infection and could explain in part the hyper-activation of the immune system.

P.D4.05.14

p.p1 {margin: 0.0px 0.0px 0.0px 0.0px; font: 11.0px Arial} The long pentraxin PTX3 has a non-redundant role in the control of *Streptococcus pneumoniae* invasive infections

R. PORTE*[1], R. Parente[1], M. Sironi[1], F. Pasqualini[1], T. van der Poll[2], C. Garlanda[1], B. Bottazzi[1], A. Mantovani[1];*
[1]Humanitas Cinical and Research Center, Pieve Emanuele (Milan), Italy, [2]Academic Medical Centre, Amsterdam, Netherlands.

Pentraxin 3 (PTX3) is a fluid phase pattern recognition molecule which has served as a paradigm for linking the cellular and humoral arms of innate immunity. PTX3 is an important component of host resistance to pulmonary infections for selected pathogens. Our aim was to investigate the role of PTX3 in the control of pneumococcal infections caused by *Streptococcus pneumoniae*, the most common causative bacteria in community-acquired pneumonia and an important cause of mortality world-wide. By using a model of invasive pneumococcal infection in young-adult mice, we observed a strong expression of PTX3 by non-hematopoietic cells. Comparing the pneumococcal load and survival of infected mice, we observed a higher sensitivity *of Ptx3*$^{-/-}$ animals during the invasive phase of the infection which could be restored by a systemic administration of recombinant PTX3. Infected *Ptx3*$^{-/-}$ mice also showed an increased inflammatory profile. Furthermore, the local exogenous instillation of PTX3 during the ongoing infection was able to reduce the expression of numerous inflammatory cytokines and the pulmonary pneumococcal load. We also observed that PTX3 specifically bind on *S. pneumoniae* but not in physiological conditions found during *in vivo* infection. The mechanism of the protective function of PTX3 remains to be fully elucidated. Our results suggest a non-redundant role of PTX3 in the control of *S. pneumoniae* infections. As inflammation and coagulation are important during pneumococcal invasive diseases, we are now studying the involvement of PTX3 in these systems for control of the infection.

P.D4.05.15

High incidence of primary immunodeficiencies in patients hospitalized for invasive pneumococcal diseases

E. Hernández-Brito[1], E. Colino[2], M. García-Luzardo[2], M. T. Martínez-Saavedra[1], F. Artiles-Campelo[3], M. Santana-Hernández[2], N. Gonzalez-Quevedo[1], ***C. Rodríguez-Gallego****[1];*
[1]Department of immunology, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas, Spain, [2]Department of pediatrics, Hospital Universitario Materno-Infantil, Las Palmas de Gran Canaria, Las Palmas, Spain, [3]Department of Microbiology, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Las Palmas, Spain.

Introduction.*Streptococcus pneumoniae* is a leading cause of bacterial pneumonia, meningitis, and sepsis in children. Some primary immunodeficiencies (PIDs) confer predisposition to invasive pneumococcal disease (IPD). **Methods.** Identification of pediatric (younger than 14 years) patients with IPD between January 2000-February 2017 from the province of Las Palmas (one million inhabitants). Clinical and epidemiologic data and immunological explorations. **Results.**We identified 186 children who suffered from IPD, of whom 68 patients (mean age 32 months; range, 0 days-13 years) required hospitalization. Fourteen of the 68 children (20,6%) had classical risk factors. Immunological evaluation could be performed to 36 patients. Seven patients suffered from a genetically confirmed PID: IRAK-4 deficiency (1 patient), X- linked agammaglobulinemia (1), congenital asplenia (2), Ataxia-telangiectasia (1), DiGeorge Syndrome (1), and Charge Syndrome (1). Oneonly patient with PID (IRAK-4 deficiency) had suffered from recurrent IPD or previous hospitalizations, and only one patient with PID (DiGeorge syndrome) developed severe respiratory infections after diagnosis. In addition, a patient with partial Chromosome 16 trisomy and recurrent pneumonias had low numbers of switched-memory B cells and high numbers of CD21^{low} B cells, and one patient, whose brother had been diagnosed with STAT3 negative AD Hyper IgE syndrome, had high IgE levels (996.00 UI/mL) and eosinophilia. **Conclusions.** PID may be the cause of 20% of pediatric patients with IPD. Prompt diagnosis and treatment after one episode of hospitalization for IPD, even in the absence of previous severe and/or recurrent infections, protect against posterior severe infections in patients with PID.

P.D4.05.16

Sex and pathogen influence on monocyte activation - A special feature arising from *Entamoeba histolytica* infection

J. Sellau*[1], M. Groneberg[1], S. Hoenow[1], B. Krause[1], C. Marggraff[1], J. Diekhoff[2], H. Ittrich[2], T. Jacobs[1], H. Lotter[1];*
[1]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany, [2]Department and Clinic for Diagnostic and Interventional Radiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

Men and male mice are more proned to develop amebic liver abscess (ALA) following infection with the parasite *Entamoeba (E.) histolytica*. In the murine model for the disease, a CCL2-dependent recruitment of Ly6C^{hi} monocytes and TNFα is responsible for tissue damage. Interestingly, in *E. histolytica* infected human, men show higher CCL2 levels compared to women suggesting a similar mechanism underlying the disease. Here, we analyzed sex-specific characteristics of human monocytes in response to *E. histolytica*. *E. histolytica*-dependent liver destruction in mice was analyzed by magnet resonance imaging (MRI), monocytes were characterized by FACS and serum cytokines by using a multiplex assay. Human macrophages or monocytes were stimulated with various antigens (LPS, LTA, *E. histolytica*) or conditioned medium, supernatants were analyzed using multiplex assays and cells were characterized by FACS. Male mice showed larger abscess lesions, higher blood and liver CD11b$^+$Ly6C^{hi} monocyte frequencies as well as an altered cytokine profile including TNFα, CCL2 and CXCL1 compared to female mice. In human, we found TNFα and CXCL1 as being regulated in a sex dependent manner in macrophages and monocytes, respectively. Furthermore, only the stimulation with *E. histolytica* led to an increased population of CCR2-expressing CD14$^+$ monocytes in men, but not in women. Inflammatory monocytes play an important role in inducing a sex difference in the outcome of ALA in mice. We were able to generate a comparable inflammatory phenotype monocyte population in a human *in vitro* model with the same sex difference, which might be able to induce a strong immune pathology.

P.D4.05.17

Dynamics and trafficking of mGBP protein complexes within membranous compartments during infection with *Toxoplasma gondii*

N. Steffens*, L. Legewie, E. Kravets, D. Degrandi, K. Pfeffer;*
Medical Microbiology and Hospital Hygiene, Düsseldorf, Germany.

Introduction: During invasion of target cells, *Toxoplasma gondii* (*T. gondii*) creates a parasitophorous vacuole (PV). Members of the murine guanylate binding protein family (mGBPs) assemble at the cytoplasmic side of the PV and interact with this membranous compartment via yet uncharacterized mechanisms.
Objectives: This analysis aims at unravelling the molecular mechanisms by which mGBPs impair the vital functions of *T. gondii*.
Methods: Stable cell lines expressing fluorescent mGBPs were generated and infected with GFP-/mCherry-expressing *T. gondii* to analyze the dynamics of mGBPs via Confocal Live Cell Imaging. Super-resolution technologies were used to analyze the doubly transduced cell lines during infections in detail. Giant unilamellar vesicles technology (GUV) is in progress to investigate the binding capacity of mGBP2 and mGBP7 to membranes and their modulation of membrane integrity. Also, cytosolic compartments of mGBPs (vesicle-like structures, VLS) and the *T. gondii*-PV will be characterized by electron microscopy.
Results: First results suggest that mGBP7 localizes in VLS after Interferon-γ stimulation. Furthermore, mGBP7 colocalizes with mGBP3 and partially with mGBP6 and mGBP9 but virtually not with mGBP2. mGBP2 appears to recruit faster to the PV compared to mGBP7. Additionally, mGBP7, mGBP3, mGBP6 and mGBP2 are able to accumulate directly at the plasma membrane of *T. gondii*, subsequently leading to parasite death.
Conclusion: mGBPs belong to a family of GTPases that can interact with the *T. gondii*-PV and selected members directly attack the parasite membrane. The analysis of the mechanisms will help to understand Toxoplasmosis and to find new treatment opportunities in the long term.

P.D4.05.18

Immunovirological response of people living with Human Immunodeficiency Virus on highly active antiretroviral therapy in Senegal

M. D. SYLLA NIANG[1], *B. Mbengue*[1], *M. Mbow*[1], *A. Sylva*[2], *S. Atsou*[1], *R. Derwiche*[1], *T. N. Dieye*[1], *A. Dieye*[1];
[1]*University Cheikh Anta Diop of Dakar - SENEGAL, Dakar, Senegal,* [2]*General Hospital of Grand-Yoff, Dakar, Senegal.*

Introduction: The objective of this study was to evaluate the immuno-virological response of HIV-infected patients by analyzing the peripheral CD4 lymphocytes rates and viral load following highly active antiretroviral therapy (HAART).
Material and methods: A cross-sectional prospective study was conducted from January to September 2017. The study population included patients living with HIV (PLHIV) regularly followed up at General Hospital of Grand-Yoff (HOGGY). The analysis focused on socio-demographic, clinical, and biological parameters. Data were analyzed by descriptive and analytical statistical methods using Statview 5.0 software.
Results: The cohort of PLHIV was composed of 127 patients. The median age at diagnosis was 41 years. The circumstances of discovery were dominated by opportunistic infections (47,3%). HIV-1 infection was predominant (88%). The most represented therapeutic combinations were Tenolam / Efavirenz (46% of patients) and Combivir / Efavirenz (27.8%) with 44 months as average length of HAART. Following HAART, the median TCD4 counts has increased from 293 cells/µL at diagnosis to 401 cells/µL. The progression of TCD4 median was significant ($p < 0.0001$) only for PLHIV-1 under HAART. Among treated patients, 22.8% underwent an immune restoration during the follow-up whilst 30% had immunological failure.
The immunovirological discordance was 23.6%. For PLHIV with detectable viral load, there was no significant difference between the initial viral load and the viral load after treatment.
Conclusion: These data allow to optimize existing treatments and contribute, through a multidisciplinary care, to improve the patient survival.

P.D4.05.19

Ageing outweighs the impact of cART regimen on the immune restoration of virally suppressed long-term treated HIV patients

R. Emilova[1], ***Y. Todorova***[1], *N. Yancheva*[2], *D. Strashimirov*[2], *I. Elenkov*[2], *I. Alexiev*[3], *M. Nikolova*[1];
[1]*NRL of Immunology, National Center of Infectious and Parasitic Diseases, Sofia, Sofia, Bulgaria,* [2]*Specialized Hospital for Active Treatment of Infectious and Parasitic Diseases, Sofia, Bulgaria,* [3]*NRL of HIV, National Center of Infectious and Parasitic Diseases, Sofia, Sofia, Bulgaria.*

Life-long combined antiretroviral therapy (cART) is the only current strategy in HIV+patients. The factors impacting residual immune activation and long-term prognosis have not been completely elucidated. Aim: To evaluate the relative impact of cART regimen and ageing on the immune restoration in virally suppressed long-term treated HIV+ patients. Material & Methods: Data for 182 HIV+ patients (52 F, 130M), mean age 43 (24-76), on continuous cART for at least 5y, with SVR (HIVVL<2.0 $\log_{10}$copies/ml) after the second year were analyzed. Subgroups according to regimen were: INSTI-based (n=14), NNRTI-based (n=28), LPV-based (n=89), DRV-based (n=54). Percentage and absolute counts (AC) of CD4, CD8, CD4-CD8-DNT and CD4+CD8+DPT were determined at 2 and 5y of cART by flow cytometry (Multitest,FACSCantoII,BD).The impact of cART regimen, age, gender, HCV/HBV coinfection and transmission category on immune restoration was evaluated by multiple regression analysis (SPSS21). Results: CD4AC and CD4/CD8 ratio did not differ between subgroups at 2y ($p>0.05$), and increased significantly at 5y of treatment (mean 629vs.519, and 0.74vs.0.58, $p<0.0001$). CD4AC and CD4/CD8 restoration were not affected by regimen ($R^2=0.01$ and, $R^2=0.003$, $p>0.05$). Age was the most important predictor for CD4/CD8 restoration(0.12vs.0.20, $F=8.30$, $p<0.029$).However, LPV-based cART was associated with lower level of DPT reflecting low thymic activity ($F=8.76$, $R^2=0.154$, $p<0.01$). In addition, advancing age predicted a lower level of DNT regulating immune activation.(109vs.171, $F=15.02$, $p<0.01$). Age is a major factor for immune restoration in long-term treated HIV patients. Old-generation PI may directly impact the regeneration and activation of T cells and should be avoided in the elderly patients.

P.D4.05.20

Extracellular matrix destruction in Tuberculosis is regulated by an acidic inflammatory microenvironment

A. M. Whittington[1], *D. E. Kirwan*[1], *F. S. Turner*[2], *R. H. Gilman*[3], *J. S. Friedland*[1];
[1]*Imperial College, London, United Kingdom,* [2]*Edinburgh Genomics, University of Edinburgh, Edinburgh, United Kingdom,* [3]*Johns Hopkins University, Baltimore, United States.*

Background: The inflammatory microenvironment is acidic. Extracellular pH at sites of *Mycobacterium tuberculosis* (M.tb) infection nears 7.0 compared to physiological pH 7.4. Acidosis is detected by pH-sensing G-Protein coupled rectors (TDAG8, OGR1 and GPR4). Tuberculosis is characterised by marked inflammation and tissue destruction driven by host derived Matrix Metalloproteinases (MMPs). This study investigates how extracellular acidosis modulates immune responses in TB.
Methods: Transcriptome profiling of primary human monocyte-derived macrophages (MDMs) infected with virulent M.tb H37Rv at pH 7.4 or with acidosis (pH 7.0) was performed by RNA-seq. MDM expression of acidosis receptors was determined by qPCR and immunohistochemistry of biopsies from TB patients. Protein secretion by infected MDMs was measured by Luminex.
Results: Acidosis produces system level transcriptional change in M.tb infected MDMs with 2616 genes upregulated and 2919 downregulated at pH 7.0 compared with 1556 up- and 1441 downregulated at pH 7.4. Genes uniquely upregulated by acidosis have multiple immune and metabolic functions including inflamasome activation and glycolysis. The most significantly upregulated pathways by acidosis are extracellular matrix degradation pathways due to upregulation of MMPs (MMP-1, MMP-3 and MMP-10 are increased 5.2, 4.2 and 6.2 fold respectively. $p<0.01$). TDAG-8 and OGR-1 are expressed by MDMs and highly expressed in M.tb infected tissue. Acidosis increased MMP-1, MMP-3 and IL-1β secretion and suppressed TNF-α from infected MDMs. These effects are dependent upon acidosis receptor signalling.
Conclusions: The acidic microenvironment enhances tissue degradation pathways and MMP secretion in TB. TDAG-8 and OGR-1 are potential novel targets for host directed therapy in TB.

P.D4.06 Exploiting host pathogen interaction - Part 6

P.D4.06.01

Investigation the role of syntenin in Salmonella Typhimurium uptake

F. F. Ali[1], *M. Jones*[2], *F. Berditchevski*[3], *P. Monk*[4], *L. J. Partridge*[5];
[1]*Mosul Technical institute, Northern Technical University, Mosul, Iraq,* [2]*Dept. Molecular Biology & Biotechnology, University of Sheffiel;d, Sheffield, United Kingdom,* [3]*Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, United Kingdom,* [4]*Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, Sheffield, United Kingdom,* [5]*Dept. Molecular Biology & Biotechnology, University of Sheffield, Sheffield, United Kingdom.*

*Salmonella*is an infectious pathogen linked with human and animal diseases world-wide. These bacteria are characterized by the ability to infect a variety of human cells. Syntenin is a scaffolding protein that is involved in various cell functions and is reported to associate with the tetraspanin protein CD63, which has previously been implicated in the adhesion of *Salmonella*Typhimurium to human monocyte derived macrophages (MDMs). The aim of this research was to investigate association ofthe bacteria with cells that had been stably knocked down for syntenin expression.The HeLa syntenin knock down (KD) cell line was generated and used using a pLKO-sh Syntenin clone, while the control cell line was transfected with pLKO-puro vector. HeLa syntenin KD and WT control were infected with different strains of *Salmonella*Typhimurium. The bacteria were stained and the bacterial-cells association were analysed using flow cytometer. Surprisingly, the HeLa syntenin KDwere more susceptible to *Salmonella* infection than control cells; however, no significant changes were observed in early stages of *Salmonella* binding. Tetraspanin expression levels were similar for the KD and the WT cells, but stochastical optical reconstruction microscopy (STORM) analysis did indicate some differences in the distribution of CD63 molecules. Overall, our results suggest that the effects of syntenin KD on *Salmonella* infection could relate to alternative syntenin partner proteins such as syndecan.

P.D4.06.02

Toward a prognostic marker for chronic Chikungunya disease

V. R. de Oliveira[1], *L. Moraes*[1], *P. M. Casais*[2,1], *T. Silva*[1], *C. Orge*[2], *L. Pamplona*[3], *B. B. Andrade*[2], *L. A. Santos*[2], *R. Venancio*[4], *A. Barral*[2,5], *M. Barral-Netto*[2,5], *R. Khouri*[2,1], ***V. Boaventura***[2,1,6];
[1]*Federal University of Bahia, Salvador, Brazil,* [2]*Oswaldo Cruz Foundation, Salvador, Brazil,* [3]*Federal University of Ceará, Fortaleza, Brazil,* [4]*Oswaldo Cruz Foundation, Rio de Janeiro, Brazil,* [5]*Institute for Investigation in Immunology, Salvador, Brazil,* [6]*Santa Casa de Misericordia da Bahia, Salvador, Brazil.*

Introduction - Chikungunya disease (CHIKD) is an arboviroses that evolves to recurrent chronic skeletal muscle symptoms in 14,4 to 87,2% of affected patients. In order to identify prognostic markers of persistent disease, we started a cohort study in four cities during an outbreak of CHIKD in Brazil . Methods - Blood, urine and saliva samples were obtained from 233 patients with acute (<10 days) symptoms of arbovirosis. Positive PCR / IgM cases (n=132) not including co-infection with DENV or ZIKAV were clinically reevaluated after two months in order to detect chronic CHIKD. Expression of chemokines and cytokines during acute phase were determined by citofluorimetry and PCR of plasma and saliva samples, respectively. Results - Chronic CHIKD occurred in 67,7% of individuals with increased risk if edema, pruritus, rash, retroocular pain and dizziness were reported during acute phase. CHIKV or IgM level were not related to disease progression. High expression of CCL2 and CXCL10 and low levels of cytokines was observed in both plasma and saliva samples, with no difference between recovered and progressors. In saliva samples, a positive correlation was observed between expression of IL-1beta and the presence of CHIKV. Conclusion - Despite a local and systemic expression of CCL-2 and CXCL10 during acute CHIKV infection, only clinical markers were found to be related to CHIKD progression to chronic stage. Positive CHIKV saliva samples exhibited an increased expression of IL-1beta, possible suggesting a CHIKV- induced activation of inflammasome. <!--EndFragment-->

P.D4.06.03

Cryptic high mannose self-recognition by macrophage Mannose Receptor leads to severe anaemia in sickle cell disease

H. Cao[1], *J. Shepherd*[1], *B. Patel*[1], *J. Brewin*[2], *A. Masson*[1], *H. Wassall*[1], *M. Forrester*[1], *E. Black*[1], *S. Leishman*[1], *A. Antonopoulos*[3], *Y. Cao*[4], *S. Henderson*[5], *G. Konieczny*[1], *H. Wilson*[1], *A. Dell*[3], *S. Haslam*[3], *D. Rees*[2], *R. N. Barker*[1], *M. A. Vickers*[1];
[1]*Institute of Medical Sciences, Aberdeen, United Kingdom,* [2]*Paediatric Haematology, Kings college Hospital, Denmark Hill, London, United Kingdom,* [3]*Department of Life Sciences, Imperial College London, London, United Kingdom,* [4]*Cranfield University, Cranfield, United Kingdom,* [5]*Scottish National Blood Transfusion Services, Aberdeen, United Kingdom.*

Sickle cell anaemia (SCA), the most haemolytic form of sickle cell disease (SCD), is characterised by genetic homozygosity of the haemoglobin S allele, severe anaemia and vascular pathology. Hypersensitive mass-spectrometry glycomic analysis of SCA red blood cell membrane (RBCs) reveals previously unrecognised high mannose N-glycans. Terminal-mannose specific lectins, snowdrop and daffodil, identify intense high mannose expression on SCA but not healthy RBCs, while annexin V binding indicated lack of phosphatidylserine exposure. Such high mannoses are found cryptically expressed in all RBCs, hidden with cytoskeletal protein spectrin and is exposed in discrete patches upon oxidation, as shown by super resolution microscopy. Strong clinical correlations between mannose exposure and markers of haemolysis, such as haemoglobin, RBC and reticulocyte counts are observed. Lack of correlation between mannose and LDH, an intravascular haemolysis indicator, suggests mannose is specifically associated with extravascular haemolysis, which accounts for 70% of total haemolysis. Macrophage phagocytosis of SCD RBCs is almost exclusively associated with Mannose Receptor positivity. Glycans (mannan/chitin) and antibody blockade of Mannose Receptor inhibit macrophage uptake of SCD RBCs. It was suggested in 1954 that sickle cell trait conferred resistance to malaria, explaining selection for the allele. Sickle heterozygous RBCs expose extra high mannose in response to oxidation, which occurs during plasmodium infection. We propose that exposure of high mannoses acts to recruit clearance by the innate immune system in the form of splenic macrophages through the Mannose Receptor. Manipulation of this high mannose recognition may assist future treatment of both SCD and malaria infection.

P.D4.06.04

Helminth-specific CD4$^+$ T cell responses during *Ascaris* infection in the pig

F. Ebner, *J. Schlosser, L. Tedin, S. Hartmann;*
Institute of Immunology, Center for Infection Medicine, Department of Veterinary Medicine, Berlin, Germany.

The pig represents the ideal human-relevant research model to study the immune response to *Ascaris* spp., the most common of the soil-transmitted helminths. The large roundworms infecting humans and pigs, *A. lumbricoides* and *A. suum,* are genetically almost identical and cross-transmission occurs, highlighting the role of pig studies to understand the host-parasite interplay. The quality of an immune reaction largely depends on a relatively small fraction of antigen-specific CD4$^+$ T cells that orchestrate the adaptive immune response. Our study therefore aimed to investigate frequency, phenotype and specificity of *Ascaris*-reactive CD4$^+$ T cells in the pig as a natural host. We used CD40L (CD154) expression as an early TCR activation marker of swine CD4$^+$ T cells to study the development of an *Ascaris*-specific CD4$^+$ T cell pool. Our data demonstrates the onset of a robust, antigen-specific Th2 response already during larval tissue migration. *Ascaris*-specific CD4$^+$ T cells are directed against excretory-secretory proteins and parasite lysates. To improve functional analysis of antigen-specific lymphocytes we adapted the method of antigen-reactive T cell enrichment (ARTE) to porcine, parasite-specific CD4$^+$ T cells. Enrichment analysis revealed phenotypic differences in CD4$^+$ T cells from peripheral *vs.* migration affected compartments, such as lung parenchyma. Our approach thereby offers a novel strategy to identify and characterize *Ascaris*-specific CD4$^+$ T cells directly in the pig, and will be used to unravel mechanisms of protection and protective antigens.

P.D4.06.05

A comparison of selected immunological parameters in cyclophosphamide treated C57BL/6 and C3H mice immunized with BCG or rBCGmIL-18

M. Wlodarczyk, A. Bednarek, J. Kowalska, M. Bromirski, M. Druszczynska, M. Kowalewicz-Kulbat, A. Krupa, W. Rudnicka, ***M. Fol****;*
Department of Immunology and Infectious Biology, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland.

The work addressed the question of potentially superior immunogenicity of recombinant *M. bovis* BCG producing mouse IL-18 compared to the parent strain BCG under immunosuppressive conditions in the context of different sensitivity of mice strains to mycobacterial infection (susceptible C57BL/6 and resistant C3H mice). Mice were intradermally immunized with BCG or rBCGmIL-18 and after 6 weeks intraperitoneally treated with cyclophosphamide (CP, 50μg/g b.w.) for 7 days. The following specimens were isolated: serum - to measure the concentration of selected cytokines (Bio-Plex Pro™ Assay), alveolar macrophages - to investigate the effectiveness of phagocytosis (acridine orange staining), bone marrow cells (immunofluorescently stained cytospins) - to estimate the expression of CD34 and CD117. The mean percentage of alveolar macrophages involved in phagocytosis, isolated form control C57BL/6 mice, both immunocompetent and immunosuppressed was similar, and it was significantly higher than in control C3H mice. Both BCG and rBCGmIL-18 immunization caused a decrease in the phagocytic activity of macrophages, however the increase in the killing effectiveness was observed. C3H mice were more efficient producers of IL-2, IL-10, IL-12 and GM-CSF, but not TNF-α, than C56BL/6 mice. The impact of immunosuppression or type of BCG strain was moderate at most and ambiguous. There were not significant differences regarding the level expression of CD34 as well as CD117 on bone marrow cells between the mouse strains. The use of CP and recombinant BCG strain did not significantly affect the expression of the receptors. This work was supported by the National Science Centre (Poland) under Grant number 2013/11/B/NZ6/01304.

P.D4.06.06

Complex interplay between Ly49 receptors and cytomegalovirus

V. Juranic Lisnic[1], *J. Zeleznjak*[1], *B. Popovic*[1], *B. Lisnic*[1], *M. Babic*[1], *M. Cesarec*[1], *A. Halenius*[2], *L. Doelken*[3], *A. Krmpotic*[1], *S. Jonjic*[1];
[1]*Faculty of Medicine, Rijeka, Croatia,* [2]*Institute of Virology, Universitatsklinikum, Freiburg, Germany,* [3]*Institute of Virology, Wurzburg, Germany.*

Cytomegaloviruses downregulate MHC I from the cell surface in order to avoid recognition by T cells. Since this strategy could trigger the NK cell-mediated "missing-self" recognition, murine cytomegalovirus (MCMV) encodes m04/gp34, a protein which brings a portion of MHC I molecules back to the cell surface enabling them to engage inhibitory Ly49 receptors (iLy49s). However, m04 brings only a small portion of MHC I to the surface. We have identified and characterized 11kDa viral protein MATp1 encoded by the MCMV's most abundant transcript (MAT) that helps in the m04-mediated MHC I surface rescue and strengthens the interaction between inhibitory Ly49 receptors and their MHC I ligands. Viruses lacking this protein are attenuated in vivo in mice of various MHC I haplotypes in an NK cell-dependent manner. Interestingly, ORF encoding this protein is highly variable among different virus strains which has prompted us to investigate whether it has been under strong selection pressure by the immune system. Differential sensitivity of various mouse strains to MCMV has been linked to the capacity of NK cells to recognize infected cells via activating Ly49 receptors and we and others have previously shown that activating Ly49P receptors require MHC I, m04 and additional virally encoded factor. Using multiple MCMV mutants we show that the unknown virus factor is the same MAT encoded protein involved in the recognition by inhibitory Ly49 receptors. Thus, MATp1 evasion of inhibitory Ly49 receptor has prompted the evolution of activating Ly49 receptors.

P.D4.06.07

Chitinase 3-like 1 protein plays a critical role in RSV-induced airway inflammation

M. Kim[1], *D. Shim*[1], *H. Cha*[1], *K. Moon*[1], *C. Yang*[1], *K. Kim*[1], *J. Park*[1], *C. Lee*[2], *J. A. Elias*[2], *M. Sohn*[1], *J. Lee*[1];
[1]*Yonsei University College of Medicine, Seoul, Korea, Republic of,* [2]*Brown University, Providence, United States.*

Background: Chitinase 3-like 1 protein (CHI3L1) (YKL-40 in humans and breast regression protein [BRP]-39 in mice) is required for optimal allergen sensitization and Th2 inflammation in various chronic inflammatory diseases including asthma. However, the role of CHI3L1 in airway inflammation induced by respiratory viruses has not been investigated. The aim of this study is to investigate the relationship between CHI3L1 and airway inflammation caused by respiratory syncytial virus (RSV) infection. Methods: We measured YKL-40 levels in human nasopharyngeal aspirate (NPA) from hospitalized children presenting with acute respiratory symptoms. Wild-type (WT) and BRP-39 knockout (KO) C57BL/6 mice were inoculated with live RSV (A2 strain). Bronchoalveolar lavage fluid and lung tissue samples were obtained on day 7 after inoculation to assess lung inflammation, airway reactivity, and expression of cytokines and BRP-39. Results: In human subjects, YKL-40 and IL-13 levels in NPA were higher in children with RSV infection than in control subjects. Expression of BRP-39 and Th2 cytokines, IL-13 in particular, were increased following RSV infection in mice. Airway inflammation caused by RSV infection was reduced in BRP-39 KO mice as compared to WT mice. Th2 cytokine levels were not increased in the lungs of RSV-infected BRP-39 KO mice. BRP-39 regulated M2 macrophage activation in RSV-infected mice. Additionally, treatment with anti-CHI3L1 antibody attenuated airway inflammation and Th2 cytokine production in RSV-infected WT mice. Conclusions: These findings suggest that CHI3L1 could contribute to airway inflammation induced by RSV infection. CHI3L1 could be a potential therapeutic candidate for attenuating Th2-associated immunopathology during RSV infection.

P.D4.06.08

Targeting Inhibitory Receptors LAG-3 and TIM-3 to Enhance Anti-parasitic CD4⁺ T cell Responses in Visceral Lesihmaniasis

R. Kumar[1], *N. Singh*[2], *B. Singh*[2], *C. Engwerda*[3], *S. Sundar*[2];
[1]*Institute of Science, Banaras Hindu University, Varanasi, India,* [2]*Institute of Medical Science, Banaras Hindu University, Varanasi, India,* [3]*QIMR Berghofer Medical Research Institute, Brisbane, Australia.*

Introduction: CD4⁺ T cells exhaustion is a common phenomenon during chronic visceral leishmaniasis (VL) which causes reduced IFN-γ secretions, critical for controlling the parasite replication. This can be mediated by abnormal expression of immunosuppressive receptors LAG-3 and TIM-3 on the surface of CD4⁺ T cells. The present study aims to investigate the role of LAG-3 and TIM-3 in patients with VL. **Methods:** Peripheral blood mononuclear cells (PBMCs) were collected from VL patients before and after drug treatment. CD4⁺ T cells were enriched using magnetic beads. Ex-vivo mRNA expression of TIM-3 and LAG-3 was measured in both PBMCs as well as enriched CD4⁺ T cells by Real-Time PCR and surface expression were examined by flow cytometry. To know the functional relevance of LAG-3 and TIM-3, whole blood cells of VL patients were stimulated for 24 hours with soluble leishmania antigen in presence of anti LAG-3 or anti TIM-3 antagonistic antibodies or isotype controls and IFN- γ secretion was measured. **Conclusion:** We observed enhanced mRNA expression of TIM-3 and LAG-3 in whole PBMCs as well as CD4+ T cells of VL patients in pre treatment stage compared to post treatment as well as enhanced surface expression as revealed by flow cytometry analysis. We observed an enhanced IFN- γ secretion in whole blood culture after LAG-3 blockade compared but there was no any effect of TIM-3 blockade on IFN- γ secretion. These results identify LAG-3 as an important immunotherapeutic target to enhance anti-parasitic CD4⁺ T cell response and treat VL patients.

P.D4.06.09

Protocadherin-1 in Respiratory Syncytial Viral bronchiolitis

K. KUMAWAT[1,2], *U. Brouwer*[3,4], *S. Fens*[1], *A. Petersen*[3], *B. Lambrecht*[5,6], *M. Nawijn*[3,4], *G. Koppelman*[3,4], *L. Meyaard*[1,2], *L. Bont*[1];
[1]*University Medical Center Utrecht, Utrecht, Netherlands,* [2]*Oncode Institute, University Medical Center Utrecht, Utrecht, Netherlands,* [3]*University Medical Center Groningen, Groningen, Netherlands,* [4]*Groningen Research Institute for Asthma and COPD, University Medical Center Groningen, Groningen, Netherlands,* [5]*VIB Center for Inflammation Research, Ghent, Belgium,* [6]*Ghent University, Ghent, Belgium.*

Early life Respiratory syncytial viral (RSV) bronchiolitis is linked to declined lung function and development of asthma in later life. SNPs in Protocadherin-1 (PCDH1), an airway epithelial surface protein, have been associated with increased susceptibility to airway hyperresponsiveness (AHR). Since aberrant AHR indicates diminished lung function and is a key feature of RSV bronchiolitis, we investigated whether PCDH1 could play a role in RSV disease.
Human bronchial epithelial cell line, 16HBE, was infected with RSV-A2 and PCDH1 expression was measured by qPCR and western blotting. C57BL/6 wild-type (WT) and PCDH1 knock-out (KO) mice were infected with RSV-A2. Body weight loss was monitored as clinical parameter for RSV disease. On 5-day post-infection (dpi), lung inflammation was determined by broncho-alveolar (BAL) fluid analyses and lung function was measured by FlexiVent.
In vitro, RSV-A2 infection decreased PCDH1 protein expression in 16HBE cells with variable effects on mRNA. *In vivo*, RSV-A2 infection led to body weight loss and showed increased viral titres in the BAL fluid, indicating an ongoing RSV disease. Moreover, BAL fluid showed significantly increased cellular influx to the lungs. Furthermore, RSV infection augmented AHR in response to methacholine. There were no differences between WT and KO mice in weight loss, lung inflammation, AHR or viral replication post-RSV infection.
In conclusion, we confirm RSV causes airway inflammation and hyperresponsivenees in adult mice, but these effects are independent of PCDH1. Ongoing studies aim to define the role of PCDH1 in the pathogenesis of RSV-enhanced allergic airway inflammation.
Funding: Supported by a LongFonds Grant.

P.D4.06.10

Adaptation to complex host niches drives resistance to neutrophils by fungal pathogen *C. albicans*

J. Lopes[1,2], *E. Backman*[1,2], *S. Holmberg*[1], *M. Stylianou*[3], *J. Jass*[3], *R. Claesson*[4], *C. F. Urban*[1,2];
[1]*Department of Clinical Microbiology, Umeå University, Umeå, Sweden,* [2]*The Laboratory for Molecular Infection Medicine Sweden (MIMS)., Umeå, Sweden,* [3]*School of Science and Technology Örebro University, Örebro, Sweden,* [4]*Section Molecular Periodontology, Department of Odontology, Umeå University, Umeå, Sweden.*

Immune systems have developed to prevent harm inflicted by other organisms. As a consequence, successful colonizers have evolved traits to escape from immune attack. *Candida albicans* is a successful colonizer of humans and its virulence is connected to the ability to adapt to stress or nutrient scarcity within the host. This environment shapes the niche for *C. albicans*. The yeast has evolved to anticipate environmental clues by predicting and adapting to secondary stimuli, thereby improving the organism's fitness. In this case the niche shapes the environment. Here, we describe one such event. Upon infection, neutrophils are rapidly infiltrating into the mucosal niche. Circulating neutrophils are effective phagocytes which serve as first line defense against fungal pathogens. High numbers of infiltrating cells coupled with the formation of microbial structures, such as biofilms, lead to induction of hypoxic and anoxic microniches. We have characterized the effect of anoxia on neutrophil responses encountering *C. albicans* both under the form of different planktonic morphotypes and under biofilm growth. We found that a persistent anoxic milieu did not affect neutrophil function, however, hampered neutrophil responses towards *C. albicans*. PAMP sensing and subsequent responses against *C. albicans* were reduced under anoxic conditions allowing the yeast to escape from neutrophil attack. In addition, anoxia contributed to increased fungal growth, a trait we found to be conserved in many *Candida* species. We therefore conclude that adaption to low oxygen is not only an evolutionary advantage but rather a pre-requisite for successful colonization and infection of the host.

P.D4.06.11

The Syk-Coupled C-type lectin receptors Dectin-2 and Dectin-3 are involved in *Paracoccidioides brasiliensis* recognition by human plasmacytoid dendritic cells

N. Preite[1], *C. Feriotii*[1], *B. Silva*[1], *D. Souza de Lima*[1], *A. Condino-Neto*[1], *A. Pontillo*[1], *V. Calich*[1], ***F. V. Loures***[2,1];
[1]*Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil,* [2]*Instituto de Ciência e Tecnologia, Universidade Federal de São Paulo, São José dos Campos, Brazil.*

Plasmacytoid dendritic cells (pDCs), which have been extensively studied in the context of the immune response to viruses, have recently been implicated in host defense mechanisms against fungal infections. Nevertheless, the involvement of human pDCs during paracoccidiodomycosis (PCM), a fungal infection endemic to Latin America, has been scarcely studied. However, pDCs were found in the cutaneous lesions of PCM patients, and in pulmonary model of murine PCM these cells were shown to control disease severity. These findings led us to investigate the role of human pDCs in the innate phase of PCM. Moreover, considering our previous data on the engagement of diverse TLRs and CLRs receptors in *P. brasiliensis* recognition, we decided to characterize the innate immune receptors involved in the interaction between pDCs and yeast cells. Purified pDCs were obtained from peripheral blood mononuclear cells from healthy donors and they were stimulated with *P. brasiliensis* with or without blocking antibodies to innate receptors. We demonstrated that *P. brasiliensis* stimulation activates pDCs that inhibit fungal growth and secrete pro-inflammatory cytokines and type I IFNs. Importantly, we also demonstrate that dectin-2 and dectin-3 are expressed on pDCs and appear to be involved (via Syk signaling) in the pDC-*P. brasiliensis* interaction. Moreover, *P. brasiliensis*-stimulated pDCs exhibited an efficient antigen presentation and were able to effectively activate CD4⁺ and CD8⁺ T cells. In conclusion, our study demonstrated that pDCs are involved in *P. brasiliensis* recognition and may play an important role in the innate and adaptive immunity against this fungal pathogen.

P.D4.06.12

Transcriptional profiling of *Leishmania infantum* infected dendritic cells: insights into the role of immunometabolism in host-pathogen interactions

M. Margaroni[1], *M. Agallou*[1], *D. Karagkouni*[2,3], *A. Hatzigeorgiou*[2,3], *E. Karagouni*[1];
[1]*Laboratory of Cellular Immunology, Department of Microbiology, Hellenic Pasteur Institute, Athens, Greece,* [2]*Diana-Lab, Department of Microbiology, Hellenic Pasteur Institute, Athens, Greece,* [3]*Diana-Lab, University of Thessaly, Volos, Greece.*

Leishmania parasites are the causative agents of leishmaniases, a group of diseases that range in manifestations from skin lesions to fatal visceral disease. Dendritic cells (DCs) hold a key role in orchestrating immune responses against leishmaniasis by regulating the activation of adaptive immunity. The aim of the present study was to investigate changes in the whole transcriptome of murine bone marrow-derived DCs infected with *Leishmania infantum* using next generation sequencing (RNA-seq). DCs exposure to parasite resulted in infection of almost 50% of DCs. The infected DCs were sorted using flow cytometry and whole RNA was isolated for transcriptome analysis. According to RNA-seq results, we identified 718 differentially expressed genes (DEGs) in DCs infected with *L. infantum* (352 up-regulated and 366 down-regulated). Comparative analysis of DEGs in infected DCs compared to DCs exposed to chemically inactivated parasites, revealed that twice as many DEGs were more abundant during infection demonstrating the influence of parasite infection on host gene transcription. KEGG pathway analysis revealed that metabolic pathways including glycolysis/gluconeogenesis and HIF-1 signaling were among the most significantly enriched pathways in infected DCs. On the other hand, DCs exposure to fixed parasite enhanced the expression of genes related to cytokine-cytokine receptor interaction and antigen processing. These data provided insights into the molecular mechanisms underlying *L. infantum* infection of DCs and might extend the knowledge regarding the interplay between metabolic processes and innate immune response against leishmaniasis.

This work was funded by the NSRF 2014-2020 and co-financed by Greece and the European Union (MIS 5002486)

P.D4.06.13

Target specific design and synthesis of a novel water soluble ferrocenylquinoline derivative as potential anti-leishmanial agent

D. Mukherjee[1], *S. Dey*[1,2], *M. Yousuf*[3], *S. Chakraborty*[1], *A. Chaudhuri*[4], *A. Dutta*[1], *A. Hussain*[1], *S. Chakraborty*[4], *S. Adhikari*[5], *C. Pal*[1];
[1]*Department of Zoology, West Bengal State University, Barasat, Kolkata, India,* [2]*PG Department of Zoology, Barasat Government College (Present Address), Barasat, Kolkata, India,* [3]*Biomaterials Group, Chemical Biology Division, CSIR-Indian Institute of Chemical Technology [IICT], Hyderabad, India,* [4]*Department of Microbiology, West Bengal State University, Barasat, Kolkata, India,* [5]*Department of Chemistry, University of Calcutta, Kolkata, India.*

Background: Visceral Leishmaniasis (VL), a neglected parasitic disease caused by Leishmania donovani, is responsible for severe health problems in India. Despite of significant progress in anti-leishmanial research, ultimate introduction of novel, safe and cost-effective drugs is far away from agreeable destination. This failure is majorly attributed to poor water solubility of drugs, in consequence, oral administration becomes challenging. Thus, the development of water-soluble oral drugs with low manufacturing prices remains highly desirable to treat VL.
Methods: We adopted quaternization strategy, where 7-Chloro-N-[2-(1H-5-ferrocenyl-1, 2, 3-triazol-1-yl) ethyl] quinolin-4-amine was modified to yield 7-Chloro-quinolin-4-yl-methyl-[2-(4-ferrocenyl-[1,2,3]triazol-1-yl)-ethyl]-ammonium chloride (CQFCWS) and tested the anti-leishmanial efficacy, both in vitro and in vivo (oral and intramuscular administration in BALB/c mice).
Results: CQFCWS was highly efficient at very low IC50 concentrations and was nontoxic towards host splenocytes, in vitro and in vivo. CQFCWS maintained hematopoietic bone marrow cell proliferation, in situ and did not alter Phase I and Phase II detoxification enzyme components in host liver. Immunomodulating potential of CQFCWS was confirmed by its ability to skew Th2 response towards Th1. CQFCWS did not induce drug resistance genes (MDR1 and MRPA) in L. donovani, in vitro. CQFCWS acted through a putative target and limited the expressions of L. donovani survival enzymes, trypanothione reductase and ornithine decarboxylase. To explore the binding efficiency of CQFCWS to trypanothione reductase, molecular docking followed by dynamic simulation was performed. CQFCWS could also induce apoptosis by upregulating PARP1 and downregulating SIR2 in parasites. Pharmacokinetic study regarding the bioavailability of CQFCWS revealed a short elimination half-life of the drug.

P.D4.06.14

The role of TAM receptors, and their ligand, Gas6, in resistance and susceptibility during ZIKV infection

L. G. Oliveira*, N. G. Zanluqui, C. M. Polonio, C. L. Freitas, J. S. Peron;*
University of São Paulo, São Paulo, Brazil.

Introduction. Zika virus (ZIKV) has gained worldwide attention as it has been correlated with severe fetal malformations, causing the Zika Congenital Syndrome (ZCS). However only 6-12% of mothers infected with ZIKV give birth to babies with malformations. These observations suggest that ZIKV infection during pregnancy is not deterministic for ZCS, but other susceptibility factors might be involved. The viral entry receptors are important candidates. Tyro3, Axl, Mertk (TAM receptors), and their ligands, Gas6 and Protein S are important candidates for ZIKV internalization and can facilitate viral entry by bridging viral envelope phosphatidylserine in a mechanism called *viral apoptotic mimicry*. Although, their correlation with resistance or susceptibility to infection, is largely unknown. **Objective.** Evaluate the immunobiology of TAM receptors, and their ligand, Gas6, in resistance and susceptibility to ZIKV infection. **Results.** We observed that SJL, susceptible mouse lineage, showed higher levels in mRNA expression of *Tyro3*, *Axl* and *Gas6* compared with C57BL/6, resistant lineage. In this context, we demonstrated that the combination of rmGas6+ZIKV in SJL and C57BL/6 infection increase the viral load in spleen while the use of Axl kinase blocker, R428, decrease the amount of viral particles. Interestingly, the use of rmGAS6+ZIKV led to the development of C57BL/6 affected offsprings, turning this lineage susceptible to ZCS. **Conclusion.** Our results suggest the crucial role of TAM receptors**,** and the intracellular kinase portion of Axl, during ZIKV infection. These data contribute for a better knowledge about the invasion mechanisms of ZIKV, *in vivo*, that could be involved in the ZCS.

P.D4.06.15

Virulent *Salmonella enterica*serovar Typhimurium modulates the production of neutrophils extracellular traps

B. M. Schultz[1,2]*, S. P. Muraro*[3]*, G. Fabiano de Souza*[3]*, S. Dias de Oliveira*[3]*, B. N. Porto*[3]*, S. M. Bueno*[1,2]*;*
[1]*Pontificia Universidad Católica de Chile, Santiago, Chile,* [2]*Millennium Institute on Immunology and Immunotherapy, Santiago, Chile,* [3]*Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil.*

Salmonella enterica serovar Typhimurium is an important cause of gastrointestinal diseases worldwide. To infect the host, *S.* Typhimurium has several virulence factors encoded in chromosomal cluster known as *Salmonella* Pathogenicity Islands (SPI). It has been described that *S.* Typhimurium infected cells secrete anti-inflammatory cytokines, promoting a tolerogenic environment. During this infection, the initial innate immune response triggered by the bacteria is the migration of neutrophils to the site of infection. These cells clear the infecting microorganisms through different mechanisms, such as neutrophils extracellular traps (NETs). However, this innate immune response is not enough to avoid *S.* Typhimurium dissemination. For this reason, it is important to evaluate the immune response mediated by neutrophils against *S.* Typhimurium. We isolate human blood or mice bone marrow derived neutrophils and induce the production of the NETs at different multiplicity of infection. $1x10^5$ cells were infected for 10 or 180 minutes, in order to, evaluate if the *S.* Typhimurium induce NETs in the early or classical way, respectively. We also performed inhibitions assays , to evaluate the NETs pathways production. We found that human blood and mice bone marrow derived neutrophils infected with a *S.* Typhimurium induce NETs production in a MOI dependent manner and by the classical way. Interestingly, a mutant strain of the SPI- 2 induces NETs in greater proportion. Our results suggest that *S.* Typhimurium infection induce NETs production, however, the bacteria has virulence factors that could be related with the suppression of the immune response, which favor the bacterial dissemination.

P.D4.06.16

The effect of hookworm co-infection on pneumococcal carriage and invasive disease

R. K. Shears*, L. C. Jacques, A. Law, N. French, D. R. Neill, A. Kadioglu;*
Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom.

Background and aims: Hookworm infections are highly prevalent in sub-Saharan Africa and South-East Asia, where the incidence of invasive pneumococcal disease is also high. Hookworms are gastrointestinal nematodes that naturally reside within the small intestine of their host for months or years, causing long-lived chronic infection. In addition, hookworm larvae migrate through the lung, which is associated with significant tissue damage. Chronic helminth infections promote an immunoregulatory environment, which is associated with increased numbers of T regulatory (Treg) cells and higher levels of the immunosuppressive cytokines, TGF-β and IL-10, which can alter the immune response to bystander pathogens. Resistance to pneumococcal disease is based upon a delicate balance between Treg-driven immune tolerance and pro-inflammatory responses which may clear infection but can also lead to tissue damage, providing a route for bacterial dissemination. Thus, the tissue damage caused by larval migration through the lung and the immunoregulation associated with adult hookworms in the small intestine may influence pneumococcal disease progression.
Methods: We are currently investigating the effect of hookworm (*Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus*) co-infection on pneumococcal disease progression using mouse models of pneumococcal carriage and pneumonia.
Results: Our data suggest that both lung migration and chronic gastrointestinal infection caused by these helminths lead to increased mortality in mouse pneumococcal pneumonia models and may also promote bacterial seeding from the nasopharynx to the lungs in pneumococcal carriage models.
Conclusions: These studies suggest that hookworm co-infections can worsen disease outcome in pneumococcal pneumonia and promote progression from asymptomatic carriage to invasive disease.

P.D4.06.17

Leishmania donovani: CD2 biased immune response skews the SAG mediated therapy for a predominant Th1 response in experimental infection

S. Sinha*, S. Sundaram;*
Centre for Biotechnology, University of Allahabad, Allahabad, India.

We have evaluated the effect of combining CD2 with conventional antimonial (sb) therapy in protection in BALB/c mice infected with either drug sensitive or resistant strain of Leishmania donovani with 3 $x10^7$ parasites via-intra-cardiac route. Mice were treated with anti CD2 adjunct SAG sub-cutaneously twice a week for 4 weeks. Assessment for measurement of weight, spleen size, anti-Leishmania antibody titer, T cell and anti-leishmanial macrophage function was carried out day 0, 10, 22 and 34 post treatments. The combination therapy was shown boosting significant proportion of T cells to express CD25 compared to SAG monotherapy. Although, the level of IFN-gamma was not statistically different between combination vs monotherapy ($p = 0.298$) but CD2 treatment even alone significantly influenced IFN-gamma production than either SAG treatment ($p = 0.045$) or with CD2 adjunct SAG treatment ($p = 0.005$) in Ld-S strain as well as in Ld-R strain. The influence of CD2 adjunct treatment was also documented in anti-leishmanial functions in macrophages.Unlike SAG treatment, treatment of SAG with CD2 also led to production of nitric oxide and TNF-a, resulting in resulting in most effective clearance of L. donovani from infected macrophages. Our results indicate that CD2, which can boost up a protective Th1 response, might also be beneficial to enable SAG to induce Macrophages to produce Leishmanicidal molecules and hence control the infection in clinical situation like Visceral Leishmaniasis.

P.D4.06.19

Protective effects of soluble human CD5 in experimental fungal sepsis

M. Velasco-de Andrés[1], *M. Martínez-Florensa*[1], *C. Català*[1], *I. Simões*[1], *E. Carreras*[1], *O. Zaragoza*[2], *F. Lozano*[1,3,4];
[1]*Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain,* [2]*Mycology Reference Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III (ISCIII), Majadahonda, Spain,* [3]*Servei d'Immunologia, Centre de Diagnòstic Biomédico, Hospital Clínic de Barcelona, Barcelona, Spain,* [4]*Departament de Biomedicina, Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain.*

Sepsis due to invasive fungal infections (IFIs) are an emerging problem worldwide related to the widespread adoption of aggressive immunosuppressive therapy among certain patient populations and the increasing use of invasive surgical interventions. Fungal recognition relies on a series of soluble or membrane-bound receptors (e.g. C-type lectins, scavengers or TLRs) expressed by host innate and/or adaptive immune cells, which could have therapeutic usefulness. Previous work by our group showed that the ectodomain of CD5, a scavenger-like lymphocyte-specific surface receptor, binds to and aggregates pathogenic and saprophytic fungal cells through recognition of β-glucans, a conserved constituent of fungal cell walls. Moreover, *i.p.* infusion of recombinant soluble human CD5 (rshCD5) protein showed protective effects in a mouse model of septic shock-like syndrome induced by zymosan, a glucan-rich particle from yeast. The present work confirms the *in vivo* efficacy of rshCD5 infusion in two experimental models of fungal infection caused by pathogenic fungal species, namely *C. albicans* and *C. neoformans.* Following therapeutic *i.v.* administration of rshCD5 to fungal-infected CD1 mice, significant time- and dose-dependent effects on mouse survival, body weight loss and fungal load were observed. Increased leukocyte infiltration (at expenses of relevant immune cell subsets such as NKs, cDCs, granulocytes and B cells) were also evidenced in targeted organs (namely kidney). Altogether, these results support the potential therapeutic value of rshCD5 administration in invasive fungal infections.

P.D4.07 Exploiting host pathogen interaction - Part 7

P.D4.07.01

The impact of T cell-derived Neuropilin-1 on immune cell infiltration and pathogen clearance during *Plasmodium* infection

H. Abberger, *J. Buer, W. Hansen;*
Institute of Medical Microbiology, University Hospital Essen, Germany.

Malaria is induced by the parasite *Plasmodium spp.* which, in 2016, affected 216 million people and led to 445,000 deaths worldwide. Characteristic symptoms include fever, headaches, seizures and in severe cases, malaria can lead to neurological complications. Cerebral malaria is caused by obstruction of brain vessels and disruption of the blood brain barrier resulting in peripheral immune cell infiltration into the brain. As we reported earlier, Neuropilin-1, a receptor of class III semaphorins and VEGF, mediates regulatory T cell migration into tumor tissue resulting in suppression of effector T cells accompanied by reduced anti-tumor response. Here, we aim to analyse whether T cell-expressed Neuropilin-1 also has an impact on immune cell infiltration into the brain with possible effects on destructive $CD8^+$ T cells during cerebral malaria. For this purpose, we infected T cell-specific Nrp-1-deficient mice with murine *Plasmodium berghei* parasites and studied pathogen clearance and development of cerebral malaria. In addition, we investigated immunological mechanisms within peripheral lymphoid organs, blood and brain by flow cytometry. Results from our study give further insights into immunological processes during cerebral malaria being of particular interest for the identification of new potential therapeutic targets.

P.D4.07.02

Characterization of newly established anti-human Dectin-1 monoclonal antibody

Y. Adachi, *M. Soda, K. Ishibashi, J. Tetsui, D. Yamanaka, N. Ohno;*
Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo, Japan.

Dectin-1 is a small C-type lectin receptor that recognizes fungal cell wall beta-glucan, and is responsible for host defense against fungal infection by producing proinflammatory cytokines. Recent reports suggest that the Dectin-1 contributes the development of diseases such as DSS-induced colitis and house dust mite-induced allergy. Controlling dectin-1 function may regulate the inflammatory diseases. To obtain antagonistic monoclonal antibody against human Dectin-1, we tried to prepare hybridoma clones by immunizing Dectin-1 KO mice with human Dectin-1 soluble protein as an antigen.
The newly established clone 2D9 produced mouse IgG1 kappa chain. The affinity of 2D9 to solid-phase human Dectin-1 molecule was monitored by BLItz. The association and dissociation constant were, $2.7x10^5$(1/Ms), and 9.2×10^{-4}(1/s), respectively. The KD of 2D9 was 3.4 nM. The 2D9 significantly inhibited the binding of soluble Dectin-1 to 1,3-β-D-glucan from *Candida albicans*. To compare the specificity of 2D9, various commercially available Dectin-1 monoclonal antibodies are applied. The binding of PE-labeled 2D9 to human Dectin-1-expressing lymphoma-transfectant was not blocked with 15E2 (BioLegend), 259931 (R&D), GE2 (GeneTex), but only by pretreatment with 2D9. While other commercial antibodies showed partially competed between 15E2 and 259931.
These results suggest that 2D9 is a unique antagonistic monoclonal antibody specific to human Dectin-1.

P.D4.07.03

In HIV primary infection, early cART reduced gd T cell activation but failed to restore their polyfunctionality

R. Casetti[1], *A. Sacchi*[1], *V. Bordoni*[1], *E. Cimini*[1], *C. Pinnetti*[1], *R. Libertone*[1], *A. Ammassari*[1], *A. Antinori*[1], ***C. Agrati***[2];
[1]*National Institute for Infectious Diseases "Lazzaro Spallanzani", Roma, Italy,* [2]*National Institute for Infectious Diseases, Roma, Italy.*

HIV infection alters phenotype, distribution and function of γδ T cells during HIV infection. There are no body of evidence about the impact of early cART on γδ T cells dynamics. HIV+ patients were divided into Early Primary Infection (EPI, Fiebig: II/IV) and Late Primary Infection (LPI, Fiebig: V/VI). Phenotype/functional analysis of γδ T cells were performed by flow cytometry before (T0) and 6 months (T6) post-therapy. γδ polyfunctional profile was assessed after specific antigens stimulation. Before therapy, higher frequency of Vδ1 and Vδ2 T cells was observed in LPI respect to HD. cART restored a normal Vδ2 T cell frequency but failed to normalize Vδ1 T cells in LPI. At T0, activation of Vδ1 and Vδ2 T cells was observed in both groups. Activated Vδ1 T cells positively correlated with viral load and negatively correlated with CD4 T cell count. cART significantly reduce the γδ T cell activation to level of HD. In both groups, CD107A expressing Vδ1 T cells was significantly lower than HD but was restored after cART. Polyfunctional profile of Vδ1 T cells of both groups was comparable to HD, except for CD107A+MIP1β+ Vδ1 T cell subset and therapy failed to restore that subset. At T6 a lower CD107A, IFNγ or TNFα producing Vδ2 T cells in EPI and a lower TNFα producing Vδ2 T cells in LPI was observed compared to HD. Our data show that HIV strongly impact γδ T cell immunity soon after infection and these alterations were only partially restored by therapy.

P.D4.07.04

Mass cytometry analysis reveals the landscape and dynamics of $CD32a^+$ $CD4^+$ T cells from early HIV infection to effective cART

S. Coindre[1], *N. Tchitchek*[1], *L. Alaoui*[1], *B. Vaslin*[1], *C. Bourgeois*[1], *C. Goujard*[2], *V. Avettand-Fenoel*[3], *C. Lecuroux*[1], *P. Bruhns*[4,5], *R. Le Grand*[1], *A. Beignon*[1], *O. Lambotte*[1,2], *B. Favier*[1];
[1]*CEA-Université Paris Sud 11-INSERM U1184, Immunology of Viral Infections and Autoimmune Diseases (IMVA), IDMIT Department, IBJF, DRF, Fontenay-aux-Roses, France,* [2]*Assistance Publique-Hôpitaux de Paris, Service de Médecine Interne et Immunologie Clinique, Groupe Hospitalier Universitaire Paris Sud, Hôpital Bicêtre, Le Kremlin-Bicêtre, France,* [3]*Paris Descartes University, EA 7327, Sorbonne Paris Cité, APHP, Necker Hospital, Virology Department, Paris, France,* [4]*Institut Pasteur, Department of Immunology, Unit of Antibodies in Therapy and Pathology, Paris, France,* [5]*INSERM, U1222, Paris, France.*

CD32a has been proposed as a specific marker of latently HIV-infected $CD4^+$ T cells. However, CD32a was recently found to be expressed on $CD4^+$ T cells of healthy donors, leading to controversy on the relevance of this marker in HIV persistence. Here, we used mass cytometry to characterize the landscape and variation in the abundance of $CD32a^+$ $CD4^+$ T cells during HIV infection. To this end, we analyzed $CD32a^+$ $CD4^+$ T cells in primary HIV infection before and after effective combination antiretroviral therapy (cART) and in healthy donors. We found that $CD32a^+$ $CD4^+$ T cells include heterogeneous subsets that are differentially affected by HIV infection. Our analysis revealed that Naive ($_N$), central memory ($_{CM}$), and effector/memory ($_{Eff/Mem}$) $CD32a^+$ $CD4^+$ T-cell clusters that co-express LILRA2 and CD64 activating receptors were more abundant in primary HIV infection and cART stages. Conversely, $LILRA2^-$ $CD32a^+$ $CD4^+$ T-cell clusters of either the T_N, $T_{CM,}$ or $T_{Eff/Mem}$ phenotype were more abundant in healthy individuals. Finally, an activated $CD32a^+$ $CD4^+$ $T_{Eff/Mem}$ cell cluster co-expressing LILRA2, CD57, and NKG2C was more abundant in all HIV stages, particularly during primary HIV infection. Overall, our data show that multiple abundance modifications of $CD32a^+$ $CD4^+$ T-cell subsets occur in the early phase of HIV infection, and some of which are conserved after effective cART. Our study brings a better comprehension of the relationship between CD32a expression and $CD4^+$ T cells during HIV infection.

P.D4.07.05

Matrix metalloproteinase 10 plays a role in dampening the host inflammatory response to *Salmonella* infection

K. Ehrhardt[1], *R. Kappelhoff*[2], *N. Steck*[3], *C. M. Overall*[2], *B. B. Finlay*[4], *G. A. Grassl*[1];
[1]*Institute of Medical Microbiology and Hospital Epidemiology and German Center for Infection Research (DZIF), Partner Site Hannover, Hannover Medical School, Hannover, Germany,* [2]*Department of Oral Biological and Medical Sciences, Centre for Blood Research, University of British Columbia, Vancouver, Canada,* [3]*Institute for Experimental Medicine, Christian-Albrechts University of Kiel and Research Center Borstel, Borstel and Kiel, Germany,* [4]*Michael Smith Laboratories, University of British Columbia, Vancouver, Canada.*

Salmonella cause a variety of diseases ranging from self-limiting enterocolitis to severe systemic infections. Dependent on the serovar, 2-5% of immunocompetent individuals become chronic carries thus representing a reservoir for transmission. The factors contributing to persistent *Salmonella* infection are incompletely understood. We identified matrix metalloproteinase 10 (MMP-10) among the highest upregulated proteases in the intestine during chronic *Salmonella* Typhimurium infection of 129Sv/J mice using a protease gene chip array. Upon in vitro infection a high upregulation of *Mmp10* mRNA was detected in primary bone marrow-derived macrophages. Infection of primary bone marrow-derived macrophages from MMP-10 deficient mice induced an increased proinflammatory response as observed by higher levels of MCP-1, IFN-beta, IFN-gamma, and nitrosative stress in comparison to infected wild type macrophages. While there was no difference in bacterial survival inside the cells at early points post-infection (6 hours and 1 day), we detected that *Salmonella* can survive better when MMP-10 is absent in long-term infected cells (3 days). Furthermore, filamentous growth of *Salmonella* was strongly increased in *Mmp10* deficient macrophages 3 days post infection. Filamentous growth can be caused by intracellular stress like nitrosative stress and might represent a survival strategy. Similarly to macrophages, *Salmonella* infection of primary intestinal fibroblasts from *Mmp10* deficient mice showed higher levels of MCP-1, nitrosative stress, filamentous growth, and increased intracellular survival (3 days) in comparison to infected wild type fibroblasts. In conclusion, our results show that MMP-10 plays a role in restricting *Salmonella* survival and dampening the host inflammatory response to infection.

P.D4.07.06

Regulation of gene and protein expression of critical factors in the etiology of colon cancer by the Microsporidia infection

C. Hurtado Marcos*, Y. Sáez, F. Izquierdo, S. Fenoy, C. Del Águila;*
Universidad San Pablo-CEU, Madrid, Spain.

Colon cancer is one of the most prevalent cancers in most countries and presents a health problem worldwide, with an incidence over one million new cases annually.In addition, the **International Agency for Research on Cancer** has identified that certain infectious agents are capable of inducing cancer in humans (18% of the global cancer burden). Based on this background our research is trying to demonstrate the correlation between colorectal cancer and infection by Microsporidia, that are obligate intracellular parasites that cause opportunistic infections in immunocompromised patients. In previous research, it was determined that Microsporidia modulates certain immune responses by regulating the apoptosis pathway and the cell cycle, inhibiting the activation of apoptotic proteins such as caspase-3 and p53 that is a key protein in the process of malignancy of epithelial cells in the intestine, hence the interest to analyze the possible association of colon cancer and Microsporidia. So, we have studied the possible regulation of proteins involved in the regulation of apoptosis and cell cycle, critical factors related to the development of colon cancer (APC, PTEN, TGF-Beta) by Flow Citometry and the expression of certain oncogenes (RAS, KRAS PI3k) by retrotranscription-PCR, in the microsporidia infection "in vitro" cellular models. The results obtained showed a clear correlation between the increase of the expression of the oncogenes with microsporia infection, as well as an induction of the proteins responsible for cell cycle. It will enrich the knowledge of the colon cancer etiology and the involvement of microsporidia infection in this pathology.

P.D4.07.07

Role of nutritional status and energetic/lipid metabolism on outcome of tuberculosis in mice

C. La Rocca[1], *V. Gigantino*[2], *T. Micillo*[3], *D. Faicchia*[1], *S. Bruzzaniti*[1], *C. Fusco*[3], *C. Palma*[4], *G. Matarese*[3];
[1]*CNR-IEOS, Naples, Italy,* [2]*Istituto Nazionale Tumori "Fondazione Pascale", Naples, Italy,* [3]*University of Naples "Federico II", Naples, Italy,* [4]*Istituto Superiore di Sanità, Rome, Italy.*

Tuberculosis (TB), a chronic infectious disease caused by *Mycobacterium tuberculosis* (Mtb), still causes high mortality in the world. Mtb is an intracellular pathogen mainly harbored by macrophages, which can attack innate and adaptive host immune response for its survival. Considering that, nutritional status and energetic metabolism highly influence the host immune function, here, we investigated the role played by a reduced caloric intake and the adipocyte hormone leptin, a factor linking energy expenditure, nutritional status and immune functions, in the outcome of Mtb infection. We found that caloric restriction reduced lung and spleen bacterial load and immune-mediated lung damage in infected DBA/2 mice.
Granuloma lesions, the number of foam cells, -a sign of Mtb-driven dysregulation of host lipid metabolism- the levels of leptin and pro-inflammatory cytokines/chemokines such as IFN-gamma, interleukin (IL)-1, IL-6 and CCL-4, were reduced in the lungs and granuloma of infected mice at CR. All this was also associated with a significant reduction in the lungs of mTOR (mammalian target of rapamycin) pathway expression, suggesting profound alterations in cell metabolism and functions. Moreover, spleen cells of CR mice better restricted bacterial growth when infected *in vitro* with Mtb. This capability correlated with a reduced development of foam cells and the switch to anaerobic glycolysis (Warburg effects). Our study suggests that the outcome of TB is influenced by nutritional status and host energetic/lipid metabolism; all these factors can be novel targets for host-directed therapy to control TB.

P.D4.07.08

The C-type lectin receptor CLEC12A recognizes plasmodial hemozoin and contributes to cerebral malaria development

M. K. Raulf[1,2], *T. Johannssen*[1,3,4], *S. Matthiesen*[1], *K. Neumann*[5,6], *F. Steinbeis*[3], *P. H. Seeberger*[3,4], *C. Strube*[2], *J. Ruland*[6,7,8], ***B. Lepenies***[1];
[1]*Immunology Unit, University of Veterinary Medicine Hannover, Hannover, Germany,* [2]*Institute for Parasitology, University of Veterinary Medicine Hannover, Hannover, Germany,* [3]*Max Planck Institute of Colloids and Interfaces, Potsdam, Germany,* [4]*Institute of Chemistry and Biochemistry, Freie Universität Berlin, Berlin, Germany,* [5]*Institute of Clinical Chemistry, Hannover Medical School, Hannover, Germany,* [6]*Klinikum rechts der Isar, Technische Universität München, München, Germany,* [7]*German Cancer Consortium (DKTK), Heidelberg, Germany,* [8]*German Center for Infection Research (DZIF), München, Germany.*

Malaria represents a major cause of death from infectious disease. Hemozoin constitutes a *Plasmodium*-derived product that contributes to disease progression of cerebral malaria. However, there is a gap of knowledge of how hemozoin is recognized by innate immunity. Myeloid C type lectin receptors (CLRs) encompass a large family of carbohydrate-binding receptors that act as pattern recognition receptors in innate immunity.
In the present study, we investigated whether and how CLRs contribute to *Plasmodium* recognition and antimalarial host defense. Using a CLR-Fc fusion protein library and CLR reporter cell lines, we identified the CLR CLEC12A (MICL) as a novel receptor for plasmodial hemozoin. Dendritic cell/T cell co-culture assays indicated that the CLEC12A/hemozoin interaction enhanced $CD8^+$ T cell cross-priming. Using the *Plasmodium berghei* ANKA mouse model of experimental cerebral malaria (ECM), we found that CLEC12A deficiency protected mice from ECM, illustrated by an increased survival, ameliorated clinical symptoms, and modulated T cell effector functions.
In conclusion, we have identified CLEC12A as an innate sensor for plasmodial hemozoin. This is the first study that shows a direct recognition of a *Plasmodium*-derived ligand by a myeloid CLR.

P.D4.07.09

"Classical" and "non-classical" Th1 lymphocytes in tuberculosis protection

I. V. Lyadova*, I. Y. Nikitina, A. V. Panteleev, T. V. Radaeva, Y. V. Serdyuck, T. A. Nenasheva, A. A. Nikolaev;*
Central Tuberculosis Research Institute, Moscow, Russian Federation.

Infection with *Mycobacterium tuberculosis* (*Mtb*) results in different outcomes ranging from pathogen clearance to severe tuberculosis (TB). Knowing immunological correlates associated with the protection of TB-exposed individuals against TB and TB patients against severe disease is important, but these correlates remain largely unknown. We have examined the features of $CD4^+$ T cells associated with TB protection.
We evaluated Th1, Th17, Th1Th17, non-classical Th1 (Th1*) and polyfunctional $CD4^+$ populations in TB patients (TBP) and *Mtb*-exposed healthy individuals. The populations were identified based on intracellular cytokines (IFN-γ, IL-17, TNF-α, IL-2) and surface expression of chemokine receptors; the frequencies, numbers, differentiation and "exhaustion" status of each population were determined.
The frequencies of Th17 and Th1Th17 lymphocytes were rare in both, TBP and *Mtb*-exposed healthy individuals. Compared to TBP, *Mtb*-exposed healthy individuals had: more "non-classical" $CXCR3^+CCR6^+$ Th1* lymphocytes; less "classical" $CXCR3^+CCR6^-$ Th1 lymphocytes; more polyfunctional $TNF\text{-}\alpha^+IFN\text{-}\gamma^+IL2^+$ cells; less bi-/monofunctional $TNF\text{-}\alpha^+IFN\text{-}\gamma^+IL2^-$ and $TNF\text{-}\alpha^+IFN\text{-}\gamma^-IL2^-$ cells. In both TBP and *Mtb*-exposed healthy individuals, Th1* and Th1 populations were functionally similar, but differed by their differentiation degree: Th1* population contained more low-differentiated $CD27^+$ effectors and lacked terminally differentiated $CD27^-CD28^-$ effectors. $TNF\text{-}\alpha^+IFN\text{-}\gamma^+IL2^+$ lymphocytes were less differentiated than $TNF\text{-}\alpha^+IFN\text{-}\gamma^+IL2^-$ and $TNF\text{-}\alpha^+IFN\text{-}\gamma^-IL2^-$ populations, however the latter were largely un-exhausted ($PD\text{-}1^-$).
In conclusion, the degree of T helper cell differentiation rather that their quantities or functional properties represents a potential correlate of TB protection. The results suggest that effective vaccine should avoid T cell over-differentiation and are relevant to the development of new vaccination strategies for TB control.
Supported by Grant RSF-15-15-00136.

P.D4.07.10

Mitochondrial fission is associated with mROS dependent microbicidal responses to *Streptococcus pneumoniae* in macrophages

M. MOHASIN[1,2], *H. M. Marriott*[1], *D. H. Dockrell*[1,3];
[1]*University of Sheffield, Sheffield, United Kingdom,* [2]*University of Dhaka, Dhaka, Bangladesh,* [3]*University of Edinburgh, Edinburgh, United Kingdom.*

Introduction: Pneumonia is a leading cause of infection-related death and *Streptococcus pneumoniae*, the commonest cause, accounts for approximately one million deaths in children each year. Macrophages are key effectors of innate immune responses but the precise microbicidal mechanisms used to control pneumococci are incompletely characterised. Recently, we demonstrated that macrophages use mitochondrial reactive oxygen species (mROS) as a component of the microbicidal response and mROS are important in an apoptotic programme that contributes to intracellular bacteria killing. West AP et al. reported that TLR agonists augment intracellular bacterial killing through inducing mROS. We hypothesized that mitochondrial homeostasis would be altered in response to intracellular bacteria as an important element of the microbicidal response to pneumococci.Methodology: Macrophages metabolic profiles, mROS generation, bacterial killing and fission/mitophagy were evaluated by XF24 analyser, flow-cytometry, gentamicin protection assay and confocal or electron microscopy/immunoblotting, respectively.Findings: *S. pneumoniae* significantly increased mitochondrial fission resulting in reduced mitochondrial network complexity 12 hours after bacterial-challenge, before apoptosis induction. Fragmented mitochondria were co-localised or adjacent to an E3 ligase Parkin, phagolysosomes and intracellular bacteria but LC3B was not recruited. mROS co-localized with mitochondria that had undergone fission. Fission was reversed by PI-3K inhibitor 3-methyladenine but was not altered by the Drp1 inhibitor Mdivi-1. Pneumococci also reprogrammed macrophages metabolism from oxidative phosphorylation to glycolysis. Mitochondrial fission was associated with increased mROS production and intracellular bacterial killing. Conclusions: Modulation of mitochondrial fission is a potential cellular target with which to recalibrate host innate immune responses against internalized pathogens.

P.D4.07.11

An *in vivo* biosensor identifies CD11c^{hi} monocytes as a cellular reservoir for *Leishmania major* proliferation and cell-to-cell spread at the site of infection

S. Heyde[1], *L. Philipsen*[1], *P. Formaglio*[1], *E. A. Seiß*[1], *P. Bousso*[2], *B. Schraven*[1,3], ***A. J. Müller***[1,3];
[1]*Institute of Molecular and Clinical Immunology, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany,* [2]*Dynamics of Immune Responses, Institut Pasteur, Paris, France,* [3]*Helmholtz Centre for Infection Research, Braunschweig, Germany.*

The virulence of intracellular pathogens such as *Leishmania major* (*L. major*) relies on their ability to undergo cycles of replication within phagocytes, release, and uptake into new host cells. While all these steps are critical for successful establishment of infection, neither the cellular niche of efficient proliferation, nor the spread to new host cells have been characterized *in vivo*.
Here, we used a biosensor for measuring pathogen proliferation by intravital 2-photon microscopy and multiparameter histocytometry in the ongoing infection. We found that monocyte-derived CD11c^{hi} cells constituted the main cell type harboring rapidly proliferating *L. major*. Synchronization of monocyte recruitment by adoptive cell transfer showed that these high proliferating parasites preferentially underwent cell-to-cell spread, however newly recruited host cells were infected irrespectively of their cell type or maturation state. We propose that among these newly infected cells, only CD11c^{hi} cells, most probably monocyte-derived dendritic cells, are permissive for efficient proliferation. In contrast, macrophages, monocytes and neutrophils may represent an obstacle in the cycle of *L. major* proliferation, release and infection of new cells.
Therefore, besides their well-described function for priming and activating T cell effector functions against *L. major*, monocyte-derived dendritic cells provide a reservoir for rapidly proliferating parasites that disseminate at the site of infection.
Supported by funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (ImmProDynamics, grant agreement n° 714233) and the German Research Foundation DFG (MU 3744/2-1 and SFB854-Z01/02) to A.J.M.

P.D4.07.12

RAS-blocking drugs: effect on Candida proteins and on metabolism of immune cells

K. H. Pinke[1], *R. A. da SIlva*[1], *B. L. Colombini-Ishikiriama*[1], *T. F. Garbieri*[1], *T. J. Dionísio*[1], *M. Fernandes*[1], *M. A. Buzalaf*[1], *S. H. de Oliveira*[2], *V. S. Lara*[1], *C. F. dos Santos*[1];
[1]*Bauru School of Dentistry; University of São Paulo., Bauru, Brazil,* [2]*Department of Basic Sciences, School of Dentistry, São Paulo State University-UNESP, São Paulo, Brazil.*

Growing evidences have linked activation of local renin-angiotensin system (RAS) with inflammatory conditions. Therefore, pharmacological interference on RAS components may exert an anti-inflammatory activity. Development of denture stomatitis, a *Candida*-associated inflammatory condition of denture-bearing mucosa, could also be affected by RAS-blocking drugs targeting renin due to molecular similarities with secreted aspartic proteases released by *Candida*. Considering that, we have evaluated the effects of blocking-RAS drugs on metabolism of human epithelial palate cells (HEPC), human gingival fibroblasts (HGF) and macrophages, and on production of pathogenic proteins by *C. albicans*. Human cells and *C. albicans* (SC5314 strain) were separately in vitro cultivated and incubated with losartan (50 µM) or aliskiren (250 µg/mL) by 24 hours, at 37ºC. Metabolism of human cells was evaluated by alamarblue assay by fluorescence reading at 488/600nm (excitation/emission).
C. albicans proteins were extracted from total fungal cells and subjected to proteomic analysis by shotgun strategy. Identified proteins from losartan- or aliskiren-treated fungi were compared with those obtained from untreated fungi using bioinformatics tools and *Candida*genome database. Among results, we highlighted upregulation exerted by losartan on WH11p, and downregulation induced by aliskiren on Adh1p, proteins related to morphological switching and to fluconazole resistance in *C. albicans*, respectively. On human cells, greater reductions in metabolism were observed after aliskiren treatment, especially on HEPC. Unlikely, losartan increased metabolism, mainly on macrophages. Altogether, our results suggest a possible modulation of pathological features related to DS by losartan and aliskiren.This work was supported by São Paulo Research Foundation (grant numbers: 2015/03965-2; and 2017/13202-1).

P.D4.07.13

Heat-killed *Mycobacterium tuberculosis* enhanced cytotoxicity to A549 lung cancer cells in classically activated-macrophage and tumor educated-macrophage through distinct responses

D. U. Putri[1,2], *S. M. Haryana*[3], *K. Y. Lee*[4,5];
[1]*Graduate Program, Faculty of Medicine, Yogyakarta, Indonesia,* [2]*International PhD Program in Medicine, Taipei Medical University, Taipei, Taiwan,* [3]*Department of Histology and Cell Biology, Faculty of Medicine, Yogyakarta, Indonesia,* [4]*Division of Pulmonary Medicine, Shuang Ho Hospital, New Taipei City, Taiwan,* [5]*Division of Pulmonary Medicine, Taipei Medical University, Taipei, Taiwan.*

Introduction: *Mycobacterium tuberculosis* (MTB), the causative agent of Tuberculosis (TB) disease is firstly recognized by alveolar macrophages. Low virulence-mycobacteria and/or its antigen have shown anti-tumor properties and are used as immunotherapy. However, the response of different macrophage phenotypes, especially in the presence of lung tumor, is poorly understood. Thus we utilized heat-killed MTB (HKTB) to elucidate the responses in different macrophage phenotypes. **Materials and Methods**: The classically activated-macrophage (MΦ) and tumor-educated macrophage (TEM) were derived from THP-1 cells, stimulated with heat-killed TB (HKTB), and analyzed for their phenotypes, protein quantification, and inflammatory cytokines expression. Furthermore, we co-cultured these HKTB-stimulated macrophages with A549 lung cancer cells to observe the cytotoxic activity. **Results:** HKTB enhanced killing potential to A549 cell in both MΦ and TEM models in a concentration-dependent manner. Cytokine analysis revealed an obvious increase of TNFα secretion from MΦ group, while TEM showed more IFNγ upregulation. Accordingly, addition of anti-TNFα to MΦ and anti-IFNγ to TEM reversed the observation. HKTB stimulation also induced proliferation towards the pro-inflammatory M1 phenotype in both macrophages. Furthermore, MΦ model showed subsequent increase of PI3K and AKT signaling which modulates inflammation and survival, as well as SUV39H1 which limits inflammation and may acts as a balance or fine-tune of macrophage polarization. **Conclusions:** Our results showed that HKTB stimulation increased cytotoxicity to A549 cancer cells by inducing pro-inflammatory responses in both MΦ and TEM models, suggesting an immunotherapeutic potential for lung cancer. However, further study should warrant the detailed mechanism and address any potential side effect.

P.D4.07.14

Enhanced interferon response genes in bronchial epithelial cells after *in vivo* rhinovirus challenge of asthma patients

A. Ravi, *J. Koster, A. Dijkhuis, S. Bal, P. J. Sterk, R. Lutter;*
Academic Medical Center, Amsterdam, Netherlands.

Rhinovirus (RV) induces acute worsening of asthma symptoms, which is linked to inadequate anti-viral responses. Bronchial epithelial cells (PBECs) are primary targets of RV and direct inflammatory and immune responses. We aim to determine anti-viral responses by PBECs from asthma patients *in vivo*.
Mild asthma patients (*n*=17) and healthy controls (*n*=4) were challenged with RV16. PBECs were obtained by bronchial brushes before and 6 days after RV16. The transcriptome was determined by RNAseq and correlated with clinical outcomes.
Anti-viral interferon response of 21 genes were upregulated (>3-fold) in asthmatics after RV16, but not in healthy controls. Interferon response genes correlated positively with the viral load (p=0.03) in bronchoalveolar lavage fluid (BALF) from asthmatics. The increased interferon response genes correlated with drop in forced expiratory volume$_1$ (R^2=0.25) and not with PC_{20} metacholine challenge tests, indicating its relation with lung function and not with airway hyperresponsiveness. BALF eosinophil cationic protein (ECP) levels increased (p=0.006) after RV16 in asthmatics and enhanced interferon response genes correlated positively with ECP (R^2=0.47) and percentage eosinophils (R^2=0.66) in BALF, but not with percentage neutrophils and myeloperoxidase. Ingenuity pathway analysis showed both type I and II interferons underlie interferon responses by PBECs in asthma.
Contrary to current thinking, asthmatics display marked interferon response genes *in vivo* to RV16, that relates to increased viral load, eosinophilic inflammation and loss of asthma control.

P.D4.07.15
RANKL improves macrophage-mediated immunity to *Leishmania major* infection

***T. S. Rigoni**, M. P. Cabral-Piccin, A. A. Filardy, M. F. Lopes, G. A. DosReis;*
Institute of Biophysics Carlos Chagas Filho, Rio de Janeiro, Brazil.

Macrophages are central effector cells in the immune response to *Leishmania* infection and their functional characteristics can be modulated by environmental stimuli. Whereas M1 macrophages (promoted by IFNγ and LPS) are associated with parasite control through the production of nitric oxide, M2 macrophages (promoted mainly by IL-4) are permissive to infection. Signals from the anatomical site in which macrophages are found can also control their tissue-specific functions. For example, receptor activator of nuclear factor kappa-B ligand (RANKL) is an important factor for macrophage differentiation to osteoclasts in the bones and also exerts regulation of the immune response in infections. However, the role of RANKL in macrophage functions in parasitic infections is still unknown.
Here, we demonstrated that treatment of inflammatory macrophages of B6 mice with RANKL and IFNγ increased nitric oxide (NO) production, as well as production of inflammatory cytokines (IL-12 and TNFα). In addition, we observed reduced expression of the M2 marker MGL-1 and increased IL-12 and iNOS expression in macrophages treated with RANKL only. Treatment with RANKL and IFNγ also reduced parasite load in macrophages infected with *L. major*, in an iNOS and reactive oxygen species (ROS)-dependent manner.
Together, these data suggest that RANKL *per se* and/or in cooperation with IFNγ enhances the effector activity of macrophages, by inducing M1 macrophage phenotype and promoting parasite control via NO and ROS production.

P.D4.07.16
Cathelicidins have their own unique fingerprint for antimicrobial and immunomodulatory activity

***M. R. Scheenstra**, M. Coorens, A. van Dijk, E. J. Veldhuizen, H. P. Haagsman;*
University Utrecht, Utrecht, Netherlands.

Cathelicidins are short cationic peptides, which play a crucial role during the innate immune response upon infection. Due to the combination of a strong antimicrobial effect combined with immunomodulatory capacities, they are promising alternatives to traditional antibiotics. The human cathelicidin LL-37, chicken cathelicidin-2 (CATH-2) and porcine PMAP-36 show similar antibacterial activities against *E. coli*. However, transmission electron microscopy (TEM) indicated that the mechanism used by these cathelicidins to kill *E. coli* are highly divergent, ranging from disrupting the membrane to complete lysis of the bacterial membrane. The immunomodulatory capacities of the cathelicidins were tested by stimulation of murine RAW264.7 macrophage cells with LPS in the presence of the cathelicidins. All three peptides efficiently inhibited LPS-induced macrophage activation. Using isothermal calorimetry (ITC), we were able to show that the mechanism of LPS inhibition differs greatly between the peptides. PMAP-36 is able to bind up to three LPS molecules, whereas CATH-2 and LL-37 bind only one LPS molecule. In addition, the binding of PMAP-36 and CATH-2 to LPS is very strong, whereas LL-37 only weakly binds LPS. In conclusion, although LL-37, CATH-2 and PMAP-36 are equally efficient in *E. coli* killing and inhibiting LPS-induced macrophage activation, their mode of action differ greatly. Understanding the inhibitory mechanisms of cathelicidins during LPS-induced immune activation could help avoid unwanted immune activation and sepsis.

P.D4.07.17
A single subcutaneous injection of chicken cathelicidin-2 in mice enhances the immune response against specific TLR ligands

***M. R. Scheenstra**, A. van Dijk, T. Cuperus, J. L. Tjeerdsma-van Bokhoven, E. J. Veldhuizen, H. P. Haagsman;*
University Utrecht, Utrecht, Netherlands.

Cathelicidins are short cationic peptides, containing both antimicrobial and immune modulatory activities. Previously it was shown that *in ovo* administration of a D-analog of chicken cathelicidin-2 (D-CATH-2) has a protective effect against a respiratory *E. coli* infection after hatch. D-CATH-2 treated chickens had reduced mortality, morbidity and bacterial load 7 days post infection. Similarly, a subcutaneous injection in mice of a truncated analogue of D-CATH-2, (DC-2_T), resulted in a protective effect against infection. To determine the mode of action of DC-2_T, different concentrations were administered subcutaneously in a single dose in mice. Increased numbers of monocytes in the blood of mice receiving the highest dose (10 mg/kg) were observed for up to 7 days. In addition, *ex vivo* stimulation of total splenocytes showed an increased immune response for specific TLR-agonists 24 hours post-injection, especially for the lower doses of DC-2_T (0.1 and 1 mg/kg). Bone marrow derived macrophages (BMDM), cultured 7 days after DC-2_T treatment, showed an increased activation upon stimulation with lipoproteins, indicating a prolonged effect of DC-2_T treatment. To show this was really due to DC-2_T injection, BMDM of naïve mice were treated *in vitro* with DC-2_T early in culture. Upon stimulation, the DC-2_T-treated BMDM showed a similar phenotype as the *in vivo* trained mice. In conclusion, this study showed that a single subcutaneous injection of DC-2_T has a prolonged protective effect in both chickens and mice. This suggests a promising role for derivatives of CATH-2 as alternatives to traditional antibiotics.

P.D4.07.18
ADCC antibodies correlate with reduced infection in a household model of influenza transmission

***S. A. Valkenburg**[1], N. Kavian[1], A. P. Li[1], N. H. Leung[2], L. L. Poon[2], B. J. Cowling[2];*
[1]HKU Pasteur Research Pole, Pokfulam, Hong Kong, [2]School of Public Health, Pokfulam, Hong Kong.

Immune correlates of protection need to be defined for the development of next generation influenza vaccines. Cross reactive antibodies which engage NK cells, to mediate Antibody Dependent Cellular cytotoxicity (ADCC), have been shown to be increased in older adults accounting for reduced H1N1 pandemic infection and risk of infection in a human challenge study, whilst also showing evidence for cross reactivity to avian viruses. Furthermore, human experimental challenge studies have shown a correlation with higher baseline titers and reduced risk of infection. Data on the protective role these cross reactive responses play in acquisition of infection and the severity of infection from baseline levels prior to infection is scarce. Our study reports on the context of household acquired infection in the Hong Kong community, and baseline PBMC and serum samples were collected from households reporting an index case of infection. Contacts of infected subjects were recruited and monitored for acquisition of infection. The baseline ADCC influenza-specific responses of uninfected contacts was found to be higher in magnitude and avidity to multiple influenza proteins than infected contacts, indicating a protective role of ADCC antibodies in the acquisition of influenza infection. Experiments are ongoing for the role of glycan effector expression for these responses. This study provides rare data on the context of community acquired influenza infection and the protective threshold of baseline immune responses for ADCC antibodies.

P.D4.07.19
Imaging Intracellular Pathogens Using Correlative Superresolution Electron Microscopy

***S. I. van Kasteren**;*
Leiden Institute of Chemistry, Leiden, Netherlands.

Intracellular pathogens can survive inside phagocytes, despite a powerful arsenal of anti-bacterial agents present in these cell. Understanding the interaction and survival mechanisms of these pathogens is therefore of utmost important to ensure the development of better antibiotics against these agents. To achieve this, we have developed a new imaging approach to study this interaction in detail: we can image pathogens selectively inside host cells using a technique called correlative light-electron microscopy, which overlays a fluorescent image (e.g. originating from a fluorescent protein) onto an electron micrograph to place it in the ultrastructural context of the cell. We have combined this technique with bioorthogonal pathogen labelling, which allows the labelling of the proteome of a pathogen with very small click-chemistry handles. This approach negates the use of genetic modification of the pathogen, broadening the application of the technique to unmodified pathogens (in our case Salmonella). To maximise the information obtained, we have recently combined super-resolution microscopy with electron microscopy. This way we can get information of the pathogen with 20 nm resolution and place this on the electron micrograph (with 1 nm resolution).

P.D4.08 Exploiting host pathogen interaction - Part 8

P.D4.08.01
Silent recruitment of TLR4 and MD-2 to the *Chlamydia trachomatis* serovar D inclusion in epithelial cells of the human urogenital tract

***S. Albrecht**, T. Miethke;*
Institute for Medical Biology and Hygiene, Mannheim, Germany.

Cells recognize invading microorganisms through pattern recognition receptors presented on their surface, resulting in the release of inflammatory cytokines. For example, the Toll-like receptor (TLR) family member TLR4 in combination with MD-2 and CD14 has been identified as the principle signal transducer in lipopolysaccharide (LPS) recognition. In the past, the role of TLR4 in *Chlamydia* recognition in epithelial cells of the urogenital tract has been controversially discussed. Whereas in rat prostate epithelial cells endogenous TLR4 is recruited to the *C. muridarum* inclusion, the co-localization of YFP-labeled TLR4 to the *C. trachomatis* serovar L2 inclusion in human cervix cancer cells could not be monitored. With our studies of endogenous TLR4 in human cervix cancer and bladder cancer cells infected with *C. trachomatis* serovar D we want to give this discussion a new turn.

Immunofluorescence studies of endogenous TLR4 and MD-2 in these cells revealed that TLR4 and MD-2 are recruited to the bacterium as early as 6 h p.I. At later time points (24-30 h p.I.), TLR4 and MD-2 are still associated with the bacterial inclusion. Additionally, we analyzed the localization of the intracellular TLR4 adapters MyD88, TIRAP and TRAM. We observed that MyD88 and TIRAP, but not TRAM, are recruited to the *C. trachomatis* inclusion. However, our data show that IL-6 and IL-8 production could not be induced by *C. trachomatis* infection. We assume that *C. trachomatis* recognition by TLR4/MD-2 does not lead to a pro-inflammatory signal and therefore remains silent.

P.D4.08.02
NLRP3 suppresses neutrophil-mediated innate immunity to helminth infection.

***R. Alhallaf**, Z. Agha, R. Eichenberger, L. Jones, J. Sotillo-Gallego, A. Kupz, A. Loukas, P. Giacomin;*
AITHM, Cairns, Australia.

Neutrophils are an important first line of defense against invading microorganisms but their role in immunity to large pathogens such as parasitic helminths is becoming increasingly appreciated. However, the mechanism of how these cells are activated and regulated following helminth infections is unclear. We demonstrate that rapid neutrophil recruitment to the lung is important for innate immunity to the nematode *Nippostrongylus brasiliensis* and these responses are suppressed by the NLRP3 inflammasome. NLRP3 deficient mice displayed elevated recruitment of neutrophils to the lung and enhanced protective type 2 immunity including elevated IL-4 and IL-13 expression in the lung and ,goblet cell hyperplasia in the intestine. Co-culture of sort-purified neutrophils with *Nippostrongylus* larvae resulted in killing of the parasite, potentially representing a mechanism of how these cells may provide protection against infection. Our findings suggest that neutrophils are important for regulating the early innate immune response to gastrointestinal helminth infections, suggesting that targeting NLRP3 may be a novel approach for limiting parasitic helminth health burdens.

P.D4.08.03
An in vitro study on the recruitment and differentiation of blood monocytes by splenic macrophages leading to macrophage hyperplasia during malaria

***J. J. Dalimot**[1], T. R. Klei[1], R. Arisz[1], J. Geissler[1], G. Bouyer[2], S. Egée[2], R. van Bruggen[1];*
[1]Sanquin Research, Amsterdam, Netherlands, [2]CNRS Station Biologique de Roscoff, Roscoff, France.

Malaria is still a global health and economic burden, responsible for 2 million deaths annually. *Plasmodium falciparum* is the most pathogenic Plasmodium species causing severe clinical symptoms like cerebral malaria, renal failure and lactic acidosis. The less understood pathology and main cause of infant mortality in endemic countries is severe malarial anemia (SMA). SMA is accompanied by splenomegaly and macrophage hyperplasia. Therefore, the elucidation of the mechanism facilitating the massive accumulation of phagocytes in the spleen could give an insight into the severe pathology of malarial anemia. By applying confocal microscopy and live cell imaging, we visualized the recruitment of circulating monocytes to splenic macrophages, which had been challenged with *P. falciparum* infected red blood cells (iRBCs). Moreover, the recruited monocytes show a red pulp macrophage-like phenotype characterized by the upregulation of the RPM specific transcription factor, SpiC. This process is dependent on the direct cell interaction with splenic macrophages and the erythrophagocytosis of iRBCs. Together these findings indicate the recruitment and accumulation of monocytes to the spleen upon malaria infection, thus, contributing to macrophage hyperplasia and the subsequent massive destruction of uninfected and infected red blood cells, ultimately leading to severe malarial anemia.

P.D4.08.04
Respiratory epithelial cells enhance IFN-γ production by natural killer and mucosal associated invariant T cells in response to *Bordetella pertussis*

***G. den Hartog**, M. Schijf, G. A. Berbers, F. R. van der Klis, A. Buisman;*
National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands.

IFN-γ is important for protective immunity to *Bordetella pertussis,* the causative bacterium for whooping cough. Here we identify an innate mucosal mechanism for the production of IFN-γ in the early stages of infection.
PBMCs from healthy donors were stimulated with proteins or inactivated intact *B. pertussis* (B1917) for 20h in the presence or absence of respiratory epithelial cells. Cell culture supernatants were analysed for cytokine production and cells for intracellular IFN-γ and CXCL10 production.
Stimulation of PBMCs with multiple isolates of *B. pertussis*, but not soluble ligands of *B. pertussis*, and IL-15 resulted in synergistically increased secretion of IFN-γ. Intracellular staining revealed that NK cells and mucosal associated invariant T (MAIT) cells were the main source of IFN-γ, but not CD4 or CD8 T cells. Depletion of monocytes or pDCs did not impair but rather increased the levels of IFN-γ. Purification of untouched NK cells showed that NK cells can produce IFN-γ following exposure to *B. pertussis* and IL-15 independent of other immune cells. Both levels of IFN-γ in the supernatant and intracellular IFN-γ increased when PBMCs were cultured in the presence of respiratory epithelial cells. Also, IFN-γ inducible chemokines CXCL9 and CXCL10 increased and staining showed that CXCL10 was mainly derived from epithelial cells.
We conclude that respiratory epithelial cells enhance rapid IFN-γ production by NK and MAIT cells following stimulation with *B. pertussis*. These data provide insight into immune responses to *B. pertussis* that could aid the development of therapeutic strategies.

P.D4.08.05
Rhinovirus induces an anabolic reprogramming in host cell metabolism essential for viral replication

***G. A. Gualdoni**, K. A. Mayer, A. Kapsch, K. Kreuzberg, A. Puck, F. Oberndorfer, K. Frühwirth, S. Winkler, D. Blaas, G. J. Zlabinger, J. Stöckl;*
Medical University Vienna, Vienna, Austria.

Rhinoviruses (RVs) are responsible for the majority of upper airway infections; despite their high prevalence and the resulting economic burden, effective treatment is lacking. We report herein that RV induces metabolic alterations in host cells, which offer an efficient target for antiviral intervention. Metabolomic analysis of RV infected cells revealed a critical role of glucose-mobilization from extracellular and intracellular pools -via glycogenolysis- for viral replication. The virus-induced enhancement of glucose uptake was dependent on PI3-Kinase and was accompanied by the upregulation of GLUT1 surface expression. Glucose was primarily required to attain a highly anabolic state in the infected cells including enhanced nucleotide synthesis and lipogenesis. Consistently, we observed that glucose-deprivation both from medium and via glycolysis inhibition by 2-deoxyglucose (2-DG) potently impairs viral replication. Metabolomic analysis showed that 2-DG specifically reverts the RV-induced anabolic reprogramming. In addition, treatment with 2-DG inhibited RV infection and inflammation in a murine model. Thus, we demonstrate that the specific metabolic fingerprint of RV infection can be used to identify new targets for therapeutic intervention.

P.D4.08.06
The mitochondrial sirtuins SIRT3 and SIRT5 control NLRP3 inflammasome activation and interleukin-1β (IL-1β) secretion

***T. Heinonen**[1], E. Ciarlo[1], D. Le Roy[1], J. Auwerx[2], T. Roger[1];*
[1]Lausanne University Hospital, Epalinges, Switzerland, [2]Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.

Background: IL-1β is involved in host defences against infections, but dysregulated expression of IL-1β is involved in inflammatory and metabolic disorders. The NLRP3 inflammasome, which is activated by reactive oxygen species (ROS), cleaves pro-IL-1β into mature, secreted, IL-1β. SIRT3 and SIRT5 are mitochondrial lysine deacetylases regulating the activity of ROS detoxifying enzymes. We previously showed that single knockout in SIRT3 or SIRT5 has no impact on innate immune responses. Hypothesizing that SIRT3 and SIRT5 may have compensatory activities, we assessed whether double deficiency in SIRT3 and SIRT5 impacts on inflammasome activity, IL-1β production and host defences against *Listeria* infection.
Methods: Mice were housed in SPF conditions. Bone marrow derived macrophages (BMDMs) were primed with TLR ligands and stimulated with monosodium urate (MSU) crystals to assess the production of ROS and cytokines. Mice were injected i.p. with MSU crystals and i.v. with *Listeria monocytogenes*. Peritoneal lavage and blood were collected to quantify ROS, cytokines and bacteria. Weight, severity scores and survival were registered.
Results: SIRT3/5 deficiency increased ROS production and IL-1β release by BMDMs, and ROS production by peritoneal cells. In a model of listeriosis, SIRT3/5 deficiency was associated with reduced bacterial burden, but had no significant effect on mouse survival.
Conclusion: These results suggest that SIRT3 and SIRT5 cooperate to control inflammasome activity and IL-1β production, and to protect from listeria burden. Thus, dual targeting of SIRT3 and SIRT5 may represent an attractive strategy for treating IL-1β-mediated inflammatory and metabolic diseases without increasing the risk of infection.

P.D4.08.07
Metabolic re-programming of the innate antiviral response during dengue virus infection of myeloid dendritic cells

*M. Ferrari[1], A. Zevini[1], L. Castiello[1], E. Palermo[1], M. Muscolini[1], C. Holm[2], D. Olagnier[2], **J. Hiscott**[1];*
[1]Istituto Pasteur Italia, Rome, Italy, [2]Aarhus University, Aarhus, Denmark.

Dengue virus (DENV), the leading arthropod-borne viral infection in the world, infects more than 300 million people worldwide, leading to 50,000 deaths annually. Markers associated with oxidative stress have been identified in patients with severe DENV infection, suggesting a relationship between oxidative stress and viral pathogenesis. Using genetic, biochemical and pharmacologic approaches, we demonstrated that the antioxidant gene network induced by Nrf2 transcription factor limited antiviral and cell death responses to DENV infection in primary human monocyte-derived dendritic cells (Mo-DC). Recent studies have further demonstrated that Nrf2, activated by the chemical sulforaphane (SFN) or by the Krebs cycle metabolite itaconate, dampened the release of pro-inflammatory cytokines, type I IFNs and IFN-stimulated genes, including the cGAS-STING, in response to DENV infection. Silencing of Nrf2 by RNA interference or CRISPR/Cas knockout increased both DENV infection and the associated antiviral and inflammatory responses.

As a viral evasion strategy, *de novo* DENV infection in turn targeted Nrf2 for proteasome-mediated degradation, and also down-regulated metabolic pathways involved in NADPH and glutathione synthesis, resulting in further accumulation of ROS and oxidative stress. Metabolic re-programming of the antioxidant response during DENV infection potentially establishes metabolic conditions for ROS accumulation and oxidative stress that aggravates DENV pathogenesis. Collectively, these data identify that Nrf2 and the anti-oxidant gene network as important regulators of the innate antiviral and inflammatory response, and as a target for DENV-mediated metabolic re-programming of the host response to infection.

P.D4.08.08

Immune evasion strategies of HCMV: Functional characterization of the highly polymorphic HCMV Fcγ receptor *RL12*/gp95

K. Hoffmann[1], *E. Mercé-Maldonado*[2], *H. Reinhard*[2], *E. Corrales-Aguilar*[3], *V. Khanh Le-Trilling*[4], *P. Lacher*[2], *H. Hengel*[1];
[1]*Institute of Virology, Freiburg, Germany,* [2]*Institute for Virology, Heinrich-Heine-University, Düsseldorf, Germany,* [3]*Virology-CIET, Faculty of Microbiology, San José, Costa Rica,* [4]*Institute for Virology, University Duisburg-Essen, Essen, Germany.*

Interactions of IgG with Fc-γ-Receptors (FcγRs), expressed on many immune cells, are essential for opsonization, phagocytosis and antibody-dependent cellular cytotoxicity. To avoid harmful IgG effector responses HCMV has evolved evasion strategies by expressing viral FcγRs (vFcγRs) interfering with host FcγR activation. HCMV encodes several, vFcγRs, i.e. gp34 (*RL11*), gp68 (*UL119-UL118*) and gp95 (*RL12*).
For gp34 and gp68 we have demonstrated a powerful inhibition of all activating FcγRs, i.e. FcγRI/CD64, FcγRII/CD32A and FcγRIII/CD16 . Here we focused on *RL12*/gp95 as a further potential antagonist of host FcγRs. Compared to gp34 and gp68, gp95 exhibited a remarkably different pattern of IgG-subclass-binding: while gp34 and gp68 bind readily to all human IgG-subclasses, gp95 binding is restricted to human IgG1 and IgG3. Further differences were observed with respect to gp95 efficiency to antagonize human FcγRs. Using Rituximab (anti-CD20) isotypes as a subclass-dependent reference IgG strong effects against FcγRIII/CD16 > FcγRII/CD32A/FcγRII/CD32B were noted while only minor effects on FcγRI/CD64 were seen, contrasting gp68 which invariably blocked all human FcγRs with a comparable efficiency.
Unlike *RL11* and *UL119-UL118*, *RL12* is one of the most polymorphic genes found in the HCMV genome. Phylogenetic-analysis based on 62 *RL12* sequences revealed 4 distinct genetic-clades. Most strikingly, extended low-complexity-regions (LCRs) reside in the N-terminus of all *RL12*-alleles. LCRs are known for their ability to rapidly adapt to dynamic changes. In line with our hypothesis that LCRs may thus modify the strength of gp95 inhibitory potential, substantial HCMV strain specific differences in gp95 attenuation of human FcγRIII responses were noted.

P.D4.08.09

Dynamic signatures of human lung resident memory CD8+ T cells during experimental influenza infection

S. Kar[1], *S. Paterson*[1], *S. Ung*[1], *A. Jozwik*[1], *Z. Gardener*[1], *S. Ascough*[1], *J. Maertzdorf*[2], *J. Weiner*[2], *H. Jarvis*[3], *O. Kon*[3], *S. Kaufmann*[2], *P. Openshaw*[3], *C. Chiu*[4], *PREPARE Consortium;*
[1]*1Section of Infectious Diseases & Immunity, Imperial College London, London, United Kingdom,* [2]*Max Planck Institute for Infection Biology, Department of Immunology, Berlin, Germany,* [3]*2National Heart and Lung Division, Imperial College London, St. Mary's Campus, London, United Kingdom,* [4]*Imperial College London, London, United Kingdom.*

Resident memory T (Trm) cells confer protection in murine models of influenza but have been little studied in humans. Here, for the first time, we show CD8+ Trm cell-mediated responses *in vivo* following human influenza challenge. Healthy adults (n=24) were challenged intra-nasally with influenza A(H1N1)2009. Blood and nasal samples were obtained throughout infection; 12 participants also underwent bronchoscopy for lower airway sampling at days 0, 7 and 28 post-inoculation. Thirteen volunteers (54%) were symptomatically infected while 11 remained uninfected. Symptoms peaked at day 4 and resolved by day 8 post-inoculation. CD8+ T cells in blood and bronchoalveolar lavage (BAL) were analysed by flow cytometry with tetramer-labelling of influenza-specific T cells. Antigen-specific CD8+ T cells in BAL were significantly enriched compared with blood and predominantly expressed the canonical Trm markers CD69 and CD103. Activation and proliferation peaked at day 7 before contracting to leave an enlarged memory pool. Matched blood and BAL CD8+ T cells were sorted by FACS and analysed by RNA sequencing to compare transcriptional profiles between anatomical compartments and differentially expressed genes (DEGs) over the course of infection. At baseline, >4000 DEGs were identified, reflecting the marked divergence between circulating and tissue populations. The most highly significant DEGs were subsequently investigated as potential novel markers of human lung Trm. The dynamic molecular profiles of pulmonary CD8+ Trm cells identified in this study thus enable further mechanistic understanding of Trm differentiation and lay the groundwork to enable the induction of local cell-mediated protection by future universal influenza vaccines.

P.D4.08.10

IL-26 inhibits Hepatitis C virus replication in hepatocytes

V. Larochette[1,2], *É. Beaumont*[3], *L. Preisser*[1,2], *P. Pignon*[1,2], *S. Blanchard*[1,4,2], *J. Dauvé*[5], *M. M. Poranen*[6], *A. Morel*[1,5,2], *H. Fickenscher*[7], *Y. Delneste*[1,4,2], *P. Roingeard*[3], *P. Jeannin*[1,4,2];
[1]*CRCINA INSERM U1232 Team 7, Angers, France,* [2]*Labex IGO, Angers, France,* [3]*INSERM U966, Tours, France,* [4]*Hospital of Angers, Angers, France,* [5]*ICO Paul Papin, Angers, France,* [6]*University of Helsinki, Helsinki, Finland,* [7]*University Christian Albrecht of Kiel, Kiel, Germany.*

Initially identified as a molecule overexpressed in virus-transformed human T cells, IL-26 has been classified as a proinflammatory cytokine due to its capacity to induce inflammatory cytokines production by myeloid and epithelial cells. Nevertheless, its biological functions remain largely unknown, in part because of its absence of orthologs in rat and mouse.
Our team has previously reported that IL-26, overexpressed in HCV-infected patients, activates NK cells, rendering them able to kill HCV-infected hepatocytes (Miot et al. 2015). Results also showed that IL-26 accumulates in hepatocytes of HCV-infected patients, a localization inconsistent with a classical role for a cytokine. Another study reported that IL-26 modulates in vitro the infectivity of three enveloped viruses, independently of the immune system.
In this study, we demonstrate that IL-26 protects hepatocytes from HCV infection. Confocal microscopy revealed that IL-26 colocalizes with dsRNA. This direct antiviral activity appears to rely on the capacity of IL-26 to bind to viral RNA and inhibit its replication. This study identifies a direct protective role of IL-26 against HCV infection and increases our understanding of the antiviral defense mechanisms contributing to control HCV infection.
Collectively, our studies show that IL-26 may have a dual protective role during HCV infection: (i) an indirect role via its capacity to induce NK cell-mediated death of HCV-infected cells and (ii) a direct role through its ability to inhibit viral replication.

P.D4.08.11

mGBP7 and interacting proteins in the combat against *Toxoplasma gondii* infection and emerging insights into the biochemical properties of mGBP7

L. Legewie[1], *S. Smits*[2], *N. Steffens*[1], *E. Kravets*[1], *M. Prescher*[2], *D. Degrandi*[1], *L. Schmitt*[2], *K. Pfeffer*[1];
[1]*Institute of Medical Microbiology and Hospital Hygiene, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany,* [2]*Institute of Biochemistry, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany.*

Introduction: *Toxoplasma gondii* (*T. gondii*) is an obligate intracellular parasite and the causative agent of toxoplasmosis. During host cell invasion, *T. gondii* forms an unique membranous compartment called the parasitophorous vacuole (PV). Members of the murine guanylate binding protein (mGBP) family translocate to the PVM leading to its disruption, but the underlying molecular mechanisms are poorly understood. The aim of this project is to provide a better understanding of the biochemistry of mGBPs as well as to elucidate the molecular mechanisms by which this IFNγ-inducible protein family is able to attack the parasite directly.
Methods: The GTPase activity of mGBP7 and its oligomerization status were analysed using the malachite green phosphate assay and SEC-MALS. For the identification of potential mGBP7 interaction partners originating from the host cell or the invading *T. gondii* co-immunoprecipitation (co-IP) and mass spectrometry (MS) experiments were performed.
Results: The Hill coefficient *h* of mGBP7 indicates a positive cooperativity of GTP hydrolysis and a half maximal concentration constant $K_{0.5}$ in the µM range. In accordance to the Hill coefficient the SEC-MALS results support the assumption that mGBP7 stimulates the GTPase acitivity in a cooperative manner and constitutes a very transient oligomer. The MS results on the other hand offer hints about potential mGBP7 interaction partners and reveal that mGBP7 is ubiquitinated independent of *T. gondii* infection.
Conclusions: The biochemical characterization of mGBP7 as well as the identification of potential mGBP7 interacting proteins provide new insights into the dynamic interactions at the interface of parasite and host.

P.D4.08.12

Mechanisms of immune evasion to alpha beta T cells in Cytomegalovirus infection, positively impinge on gamma delta T cells response

L. L. MASSARA[1], *C. Fieldings*[2], *H. Kaminski*[1], *J. Visentin*[1,3], *V. Pitard*[1,4], *A. Stum*[1,4], *M. Pierre*[1,3], *W. Gavin*[2], *D. Julie*[1];
[1]*ImmunoConCEpT UMR5164 CNRS, BORDEAUX, France,* [2]*Cardiff Institute of Infection & Immunity, Cardiff CF14 4XN, United Kingdom,* [3]*University Hospital of Bordeaux, Bordeaux, France,* [4]*Flow Cytometry Facility, TransBioMed Core, University of Bordeaux, Bordeaux, France.*

Human cytomegalovirus(CMV),a βHerpes virus, is considered as a paradigm for viral evasion. It is an important opportunistic pathogen in immunocompromised patients and a major cause of congenital birth defects when acquired in utero. HCMV encodes at least four US genes to prevent antigen presentation to αβ T cells through inhibition of HLA-I expression. CMV also decreases NK cell functions by mimicking or down-regulating ligands for NK receptors. However,the impact of these evasion mechanisms on γδ T cells is unknown. We studied the influence of the modulation of HLA-I expression on the reactivity of αβ versus γδ T cells. We infected cells with either HCMV wild type(CMV-WT) or HCMV lacking US encoding genes involved in HLA-I downregulation(CMV-ΔUS). CMV-ΔUS-infected fibroblasts expressed much more HLA-I than CMV-WT-infected cells. Interestingly,γδ T cell clones were activated to produce INF-γ when cultured with CMV-WT infected fibroblasts, but not when fibroblasts were infected with CMV-ΔUS.Furthermore, when isolated from the same CMV-infected patient, purified γδ and αβ T cells respond in an opposite way to CMV-WT vs CMV-ΔUS infected cells, according to the magnitude of HLA-I molecules.

We have previously reported that γδ T cells responding to CMV express high levels of killer Ig-like receptors (KIR). We are therefore currently studying the implication of KIR receptors and of the different US genes in the HLA-I dependent modulation of γδ T cell response to HCM.In conclusion,the immune escape processes evolved by CMV could promote γδ over αβ T cell response and increase our knowledge on how γδ T cells respond to the virus which is important in immunosuppressed individuals.

P.D4.08.13

C-type lectin receptor (CLR)-Fc fusion proteins as a tool to screen for novel CLR/bacteria interactions

S. Mayer[1], *R. Moeller*[1], *J. Monteiro*[1], *K. Ellrott*[2,3], *C. Josenhans*[2,4,5], *B. Lepenies*[1];
[1]*Immunology Unit & Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine Hannover, Hannover, Germany,* [2]*Institute for Medical Microbiology, Medical School Hannover, Hannover, Germany,* [3]*German Center for Infection Research (DZIF), Partner site Hannover-Braunschweig, Germany,* [4]*Max von Pettenkofer Institute, Ludwig Maximilian University Munich, Munich, Austria,* [5]*German Center for Infection Research (DZIF), Partner site Hannover-Braunschweig and Munich, Germany.*

The host is challenged every day by a huge number of various pathogens. Highly conserved microbial structures (pathogen-associated molecular patterns (PAMPS)) are recognised by pattern-recognition receptors (PRRs) localised on immune cells. Upon PAMP detection, several effector functions like phagocytosis and antigen presentation are induced. C-type lectin receptors (CLRs) represent one group of PRRs. While the interaction of CLRs with several pathogens-derived ligands has been described, still little is known about the role of CLRs in bacterial recognition.
This work describes innovative methods based on a comprehensive library of recombinantly expressed CLR-hFc fusion proteins to unravel novel CLR-bacteria interactions. For demonstration, the important human pathogens *Group A Streptococcus* and *Campylobacter jejuni* were used for exemplary studies to demonstrate that these methods can be easily applied to Gram-positive as well to Gram-negative bacteria. First, a plate-bound ELISA-based assay was established to allow for a high-throughput pre-screening of potential bacteria-CLR interactions. Furthermore, a flow cytometry-based assay was used to screen for CLR-bacteria interactions in solution and finally, confocal microscopy allowed for visualization and direct characterisation of the interaction. Using this combination of different techniques, we have identified candidate CLRs that may play a role in bacterial recognition. Our study enables new insights into the host innate immune response against the respective bacteria.

P.D4.08.14

Type I interferon induction by *Orientia tsutsugamushi* depends on nucleic acid recognition but does not require bacterial viability

Z. Orfanos[1], *V. Hefter*[1,2], *S. Bauer*[3], *C. Keller*[1,2];
[1]*Institute of Virology, Philipps University Marburg, Marburg, Germany,* [2]*German Centre for Infection Research at the Institute of Virology, Philipps University Marburg, Marburg, Germany,* [3]*Institute of Immunology, Philipps University Marburg, Marburg, Germany.*

Scrub typhus, a potentially lethal infection caused by the obligate intracellular Gram-negative bacterium *Orientia tsutsugamushi*, is a mite-borne zoonosis associated with strong cytokine induction. Upon infection, *Orientia* enters phagocytic cells by receptor-mediated endocytosis and shortly after escapes the endosome to replicate in the cytoplasm. Infection results in a strong induction of TNF-α and type I interferon within a few hours. Previous work proposed that interferon (IFN)-β is exclusively induced by live bacteria in macrophages. However, ligands and receptors responsible for this induction remain unknown.
We are investigating type I interferon induction by *Orientia* in mouse bone marrow-derived dendritic cells (BMDC). Our experiments show that live organisms induce IFN-β mRNA in C57BL/6 BMDC within hours after infection. Stimulation of BMDC with bacteria inactivated at 95°C showed a dramatically reduced IFN-β induction. Surprisingly, bacteria inactivated at a lower temperature induced as much IFN-β as live bacteria. These results point to a heat-stable ligand that is differentially accessible to innate receptors depending on the viability or degree of damage of the bacteria. The IFN-β induction was dependent on the endosomal Toll-like receptors 3, 7 and 9, suggesting that the ligand is a nucleic acid. Thus, IFN-β induction in BMDC in response to *Orientia* does not require viable bacteria but can also be recapitulated by dead organisms under certain conditions. We propose that the accessibility of nucleic acid ligands to innate receptors is different in live, dead or damaged bacteria, and that their exposure in the endosome leads to distinct type I interferon responses.

P.D4.08.15

Preparation, identification, and preliminary application of mAbs against Sao-M protein of S.suis 2

X. Pan[1], *J. Wang*[2], *X. Li*[1], *D. Hu*[1], *C. Wang*[1];
[1]*Hua Dong Research Institute for Medicine and Biotechnics, Nanjing, China,* [2]*Wuxi Maternity and Child Health Care Hospital, Wuxi, China.*

Introduction: Streptococcus suis serotype 2 (S.suis 2) has becoming a highly invasive zoonotic pathogen. In particular, two large-scale outbreaks of lethal S.suis 2 infections had emerged in China. Previous experiments showed that Sao-M was an excellent candidate for detection of S.suis 2. In this research, We prepared monoclonal antibodies (mAbs) against Sao-M and analyzed their biological characteristics. Materials and Methods: BALB/c mice were immunized three times with purified Sao-M protein plus adjuvant. Two weeks after the final immunization, mice were immunized with Sao-M alone. Then, spleen cells were isolated three days after the last immunization, and fused with SP2/0 myeloma cells using PEG. Hybridoma cells secreting mAbs were screened by continuously subculture, which were then injected into mice to produce mAbs ascites. Titers of mAbs from ascites were determined with ELISA.
Isotyping was performed with an Ig subtype ELISA kit. The ability of mAbs binding to native Sao-M was detected by incubating with S.suis 2. Results: Four hybridoma cell lines were obtained and all of them were able to secrete mAbs stably. The mAbs from the four cell lines all belonged to IgG1 subtype and the titers were all higher than 1:102,400. Analysis revealed that the mAbs could bind to Sao protein on the surface of S.suis 2 and had a significant effect on bacterial morphology.Conclusion: We prepared four mAbs against Sao-M protein successfully, which laid the foundation for rapid detection of S.suis 2 infection and the function research of Sao proteins.

P.D4.08.16

The secrets of the adaptation of *Brucella* to the hostile environment of the host

G. Potemberg[1,2], *J. Sternon*[1], *A. Demars*[1], *X. De Bolle*[1], *E. Muraille*[2];
[1]*University of Namur, Namur, Belgium,* [2]*Université Libre de Bruxelles, Bruxelles, Belgium.*

Understanding how pathogenic bacteria adapt to the specific microenvironment of the host to successfully colonize is matter of importance especially with bacteria that can evade immune system and are able to induce chronic infection. Infection by the bacteria *Brucella* results in common chronic disease in humans and animals called brucellosis. Actually with still no efficient vaccine available brucellosis represents huge economical losses. Moreover chronicity and recurrence of this infection cause significant morbidity despite heavy antibiotic treatments.
To better understand *Brucella* infectious cycle in mice lungs after intranasal infection, we used in situ visualization approach of the bacterial multiplication with fluorescent reporter system. Our observations by microscopy suggest that replicative bacteria are more sensitive to the immune response. A fraction of the non growing bacteria that survive the first 24h later give rise to an intensive proliferation in some alveolar macrophages. This non replication strategy may reflect adaptation to host hostile environment.
Here we have identified a set of genes that are essential for colonization of mice lungs by high density transposon insertion site sequencing Tn-Seq on the whole genome of *Brucella melitensis*. Using this method at selected time point during the first 48 hours of *Brucella* pulmonary infection, our analysis show that efficient host colonization by *Brucella* not only rely on virulence gene expression but also on important adaptation of the metabolism to the divers environment encountered during infectious cycle. Identification of essential genes for bacterial survival in context of pulmonary infection may lead to more effective prophylactic strategies.

P.D4.08.17

Human and bacterial antimicrobial peptides activate signal inhibitory receptor on leukocytes-1

M. Rumpret[1], *M. van der Linden*[1], *J. van Strijp*[2], *N. M. van Sorge*[2], *L. Meyaard*[1];
[1]*Laboratory for Translational Immunology, University Medical Centre Utrecht, Utrecht, Netherlands,* [2]*Department of Medical Microbiology, University Medical Centre Utrecht, Utrecht, Netherlands.*

Bacteria of the genus *Staphylococcus* commonly colonize humans and although closely related, comprise species with vastly different pathogenic potential. This makes *Staphylococci* a good model to study differential immune responses to commensals and pathogens. Signal inhibitory receptor on leukocytes-1 (SIRL-1) is a negative regulator of anti-microbial and inflammatory functions of myeloid cells. Phenol-soluble modulins (PSMs) are short amphipathic peptides secreted by different staphylococci. Structurally, they are very similar to the human antimicrobial peptide cathelicidin LL-37. We found that *S. aureus* is able to activate a SIRL-1-reporter cell line and using a transposon library screen, identified PSMs as potential ligands. Knock-outs of PSMs in *S. aureus* failed to activate SIRL-1 reporter cells, which was rescued by re-introducing PSM by plasmid complementation in two different *S. aureus* strains. Moreover, synthetic PSMs, as well as synthetic human LL-37, activated SIRL-1 reporter cells, further demonstrating that staphylococcal PSMs and human LL-37 ligate SIRL-1. We propose that the SIRL-1 / LL-37 interaction is in place to limit immune cell activation or restore immune homeostasis after clearance of the microbial threat. Further, pathogenic *Staphylococci* may exploit SIRL-1 as an immune evasion strategy in the context of skin and soft tissue infections, which are frequently caused by *S. aureus* and related PSM-producing *Staphylococci*.

P.D4.08.18

Human Epidermal Langerhans cells might constitute an underestimated HIV reservoir

***S. Saluzzo**, J. Strobl, N. Bayer, A. Rieger, V. Touzeau-Roemer, G. Stingl, G. Stary;*
Medical University of Vienna, Vienna, Austria.

Introduction: Viral reservoirs are major obstacle to HIV eradication. The skin is a highly immunologically active organ and containing CD4+ T cells and Langerhans cells (LCs), the major targets of HIV infection. LCs can restrict HIV viral replication and are thought to be determinant in reducing the risk of HV transmission at mucosal level. However, recent evidence suggests that also antigen presenting cells (APCs) could remain latently infected with HIV upon establishment of a chronic infection. Our project aims at understanding if the skin resident cells could harbor latently infected cells and eventually provide new insides in the mechanisms of HIV latency. **Methods:** We collected skin biopsy from individuals during the viremic phase of HIV infection as well as under antiretroviral therapy (ART) with undetectable viral load (ndVL). We investigated HIV latency analyzing p24 expression by Tissue-FACS software and electron microscopy (EM). **Results:** IF staining showed the presence of a $CD4^+$ $CD3^-$ epidermal immune cell population still harboring p24 expression both in viremic patients and those with ndVL. We identified this population as being exclusively composed by LCs. **Conclusions and future prospects:** Although preliminary, our results suggest that LCs might still harbor HIV virions in individuals with suppressed viral replication. Further analysis will prove if $p24^+$ cells contain HIV RNA and DNA by RT-PCR as well as RNA and DNA Scope analysis and electron microscopy. If this is the case, our experiments will focus in understanding infected LCs could present replication competent virus and constitute an underestimated HIV reservoir.

P.D4.08.19

The Use of CRISPR-Cas9 Based Genome-Scale Screening for Mapping the Intracellular Immune Response of NK Cells Against Lentiviral Gene Delivery

***A. Sarac**[1,2,3], E. Sayitoglu[4], A. E. Eyupoglu[1,2], D. Ozkazanc[1,2], B. Erman[2,1], A. D. Duru[4], T. Sutlu[1];*
[1]Nanotechnology Research and Application Center, Sabancı University, İstanbul, Turkey, [2]Faculty of Engineering and Natural Sciences, Sabancı University, İstanbul, Turkey, [3]Genetic Engineering and Biotechnology Institute, TUBITAK, MAM, Kocaeli, Turkey, [4]NSU Cell Therapy Institute, Nova Southeastern University, Fort Lauderdale, United States.

Immunotherapies based on Natural Killer (NK) cells show promise in the treatment of cancer. However, the biological and practical challenges of viral vector based gene delivery to NK cells significantly reduces clinical possibilities. There is scarce information about the intracellular immune response pathways triggered by viral vector entry. Anti-viral responses have been studied thoroughly in wild-type virus infections but the field has been mostly overlooked from the perspective of gene therapy vectors. In this study, we used the genome-wide screening ability of CRISPR/Cas9 system to discover mechanisms taking part in resistance to lentiviral gene delivery.
We utilized Genome-scale CRISPR Knock-out Libraries (GeCKO) in addition to next-generation sequencing technologies to screen genes taking part in these responses. By means of the controlled experiment set-up, we were able to determine that RIG-I/MDA5 and certain members of Toll-like receptor families (TLRs) are candidate pathways which play a crucial role in the response to lentiviral vector entry, triggering the secretion of antiviral factors in NK cells. Our data suggest that TLR or RLR mediated detection of viral vector components activates an anti-viral response, negatively affecting the efficiency of lentiviral gene delivery. Development of novel gene transfer protocols based on the inhibition of intracellular antiviral responses will open up the possibility to enlarge the base of cell types which can efficiently be used in gene therapy protocols.

P.D4.08.20

Estrogen Receptor Alpha agonist and antagonist differentially modulate host immunity during ascending Urinary Tract Infection

***A. Sen**[1], S. Boddu[1], J. Iyer[2], A. Kaul[1], R. Kaul[1];*
[1]Oklahoma State University Center for Health Sciences, Tulsa, United States, [2]Northeastern State University, Tahlequah, United States.

The protective role of estrogen against Urinary Tract Infection (UTI) has been well-established, but the underlying mechanisms are still unknown. Our *in vivo* studies showed that estrogen via estrogen receptor alpha (ERα) protects against Dr *E. coli* induced UTI by altering CD55 (Dr *E. coli* receptor) and TNFα expression in kidney. *We hypothesized that ERα agonist and antagonist treatment in mice modulates CD55 and TNFα expression in bladder and kidney altering UTI pathogenesis.* This study determined the effects of ERα agonist, propyl-pyrazole-triol (PPT), and ERα antagonist, methyl-piperidino-pyrazole (MPP), on inflammation and UTI outcome.
PPT or MPP pre-treated mice received Dr *E. coli* induced UTI. Bacterial load, CD55 and TNFα levels in bladder and kidney were determined. PPT and MPP showed opposite effects on these markers in bladder vs kidney. PPT treatment significantly reduced bacterial load($P<0.05$) and CD55 expression($P<0.01$) with minimal changes in TNFα levels in kidney, while in bladder, higher bacterial load correlated with increased TNFα levels($P<0.01$). In contrast, MPP reduced bacterial load and CD55 expression in bladder, but not in kidney. MPP pre-treatment in uninfected state significantly increased TNFα levels ($P<0.05$) only in bladder, resulted in better bacterial clearance and lowered the TNFα levels ($P<0.01$) upon UTI induction.
These results confirmed the protective role of ERα in kidney, however in the bladder other ERs may be involved. Our results have important clinical implications for women undergoing hormonal therapy. Also, boosting TNFα production in the bladder by drugs like MPP will serve as novel therapeutic strategy to prevent UTI recurrence.

P.D4.08.21

***In vivo* identification of cell type-specific and redundant functions of interferons in protection against influenza**

***S. A. Stifter**[1,2], N. Bhattacharyya[1,2], A. Sawyer[1], A. Sher[3], W. J. Britton[1,2,4], C. G. Feng[1,2];*
[1]The University of Sydney, Sydney, Australia, [2]The Centenary Institute, Sydney, Australia, [3]National Institute of Allergy and Infectious Diseases, Bethesda, United States, [4]Royal Prince Alfred Hospital, Sydney, Australia.

Interferons (IFNs) regulate immunity by controlling the recruitment and activation of cells, and by inducing direct anti-viral effector mechanisms via expression of hundreds of IFN regulated genes. While these effector mechanisms have been studied extensively in the context of genetically deficient mice, gene expression analysis and direct anti-viral activity *in vitro*, little is known about individual cellular responses to IFNs *in vivo*. Using a novel line of transgenic mice expressing DsRed fluorescent protein under the control of the IFN inducible gene *Irgm1* (M1Red mice), we herein characterise the response to IFN signalling at the single cell level *in vivo* following infection with influenza A virus. Although all lung-leukocytes showed a propensity to respond to IFNs, cells of the monocyte/macrophage lineage were by far the most IFN-sensitive. Unexpectedly, among pulmonary epithelial cells, alveolar type-I pneumocytes (AT-I), but not surfactant producing type-II pneumocytes (AT-II) exhibited a robust IFN-response. Conversely, AT-II demonstrated 10-100 fold higher viral gene expression than AT-I following *in vivo* IAV infection. Interestingly, although genetic deletion of IFN receptors led to a decreased IFN-response in pneumocytes, viral load was unchanged in *Ifnar1*$^{-/-}$, *Ifngr1*$^{-/-}$ *Il28ra*$^{-/-}$ or *Ifnar1*$^{-/-}$*Il28ra*$^{-/-}$ lung epithelial cells, indicating a significant redundant role in the IFN system in directing anti-viral functions. Indeed, only complete deletion of the IFN-response using *Stat1*$^{-/-}$ mice led to exacerbated early viral loads, revealing the cooperation of all three IFN systems, rather than any single one, is essential for controlling early influenza infection.

P.D4.08.22

Pre-existing AAV8 $CD8^+$ T cells are present in all donors and arbor a cytotoxic function.

***R. XICLUNA**[1], C. VANDAMME[2], M. DEVAUX[1], N. JAULIN[1], M. GUILBAUD[1], C. COUZINIE[1], J. LE DUFF[1], L. HESNARD[3], X. SAULQUIN[3], O. ADJALI[1];*
[1]Inserm UMR1089, NANTES, France, [2]Institute of clinical medicine, Kuopio, Finland, [3]UMR 1232, NANTES, France.

Recombinant adeno-associated virus (rAAV) is the most largely used viral vector for *in vivo* gene therapy. Despite promising results in preclinical and clinical studies, pre-existing immunity against the viral capsid remains a major hurdle to the efficiency and safety of AAV-based gene transfer. Particularly, pre-existing anti-AAV $CD8^+$ T lymphocytes can hamper gene transfer but their impact remains poorly defined.In our study, we previously reported the use of Tetramer-Associated Magnetic Enrichment (TAME) to analyze frequency and phenotype of AAV-specific CD8+ T cells by flow cytometry in order to set up a more sensitive and comprehensive method to detect and characterize these cells. Tetramers loaded with several AAV8 peptides allowed detection of AAV8 capsid-specific CD8+ T cells among PBMCs, without amplification, in all healthy $HLA\text{-}A2^+/B7^+$ donors tested even in absence of anti-AAV8 IFN-γ ELISpot positive response. Moreover, phenotypic assessment of the detected cells revealed a T_{EMRA} profile.To be more restrictive, we tested TAME with the immunodominant peptides described since. We also detected AAV8 capsid-specific CD8+ T cells in all tested donors but at lower frequencies.To characterize the impact of this T_{EMRA} cells, we sorted AAV8-specific $CD8^+$ T cells after TAME and we succeeded in generating AAV-specific $CD8^+$ T cell lines that were cytotoxic when faced with AAV8-loaded target cells. The dissimilarities observed between presence of AAV-specific CD8+ T cells and IFN-γ responses, highlight the need to understand the onset of pre-existing anti-AAV immunity on rAAV-based gene transfer and its impact on clinical outcome in order to develop optimal strategies.

P.D4.09 Exploiting host pathogen interaction - Part 9

P.D4.09.01
Comparison of Techniques Measuring Cellular Reactive Oxygen Species in Circulating and Tissue Rat Neutrophils

*N. Fazal, **M. Baig**;*
Chicago State University, Chicago, United States.

Objectives: Production of ROS is considered as an important neutrophil function. Neutrophils utilize oxygen radicals to kill phagocytized bacteria as well as remodeling endothelial and epithelium tissues. Neutrophils have an important role to play both in innate immune response as well as tissue-remodeling and tissue repair. There are many known techniques to ascertain production and release of ROS in cultured cells, especially neutrophils. In this study we compared the efficacy and use of different methods and determined the kinetics of intracellular and extracellular oxidants production and/or release.
Methods: We used fluorometry to determine hydrogen peroxide, Photometry to measure superoxide dismutase inhibitable reduction of Cytochrome C and Luminometry to gauge peroxidase-dependent chemiluminiscence namely (Isoluminol, Luminol and Lucigenein). We used rat neutrophils to obtain blood (circulatory) and peritoneal (tissue) to study both activated, PMA- or fMLP- or LPS-stimulated and /or un-activated cells.
Results: Our results show that all the techniques used in this study were able to measure both oxidant production as well as oxidant release in neutrophil cell culture assays. Kinetics of ROS production in studied Neutrophils show nice curves over 60-minutes assay times. Intracellular vs. Extracellular production/release of ROS were followed over every 5-10 minutes and depict different patterns of peak values, which were considered statistically significant.
Conclusions: Our studies established a kinetic cell culture assay that established base-line values of reactive oxygen species production and release, over first hour of neutrophil / cell stimulation with PMA and/or fMLP and/or LPS. These assays could determine the efficacy of therapeutic agents.

P.D4.09.02
***Cutibacterium acnes* plays a role in the maintenance of skin barrier and homeostasis**

***B. S. Bolla**[1], L. Erdei[1], G. Tax[1], E. Urbán[2], L. Kemény[1,3], K. Szabó[3];*
[1]Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary, [2]Institute of Clinical Microbiology, University of Szeged, Hungary, Szeged, Hungary, [3]MTA-SZTE Dermatological Research Group, Szeged, Hungary, Szeged, Hungary.

Our skin provides a physical barrier to separate our body from the external environment. Little is known how the cutaneous microbiota may affect these functions, so our aim is to investigate the role of *Cutinebacterium acnes* (*C. acnes*), member of the cutaneous microbiome using an *in vitro* cultured human keratinocyte cell line (HPV-KER) and 3D organotypic skin models (OS). Calcium-differentiated confluent HPV-KER cultures were treated with *C. acnes*. Barrier changes were monitored measuring transepithelial electrical resistance (TEER), performing an xCELLIgence analysis and lucifer yellow (LY) penetration assays. We also analysed the expression changes of tight junction (TJ) proteins (CLDN1, 4, OCLN and ZO-1) by western blotting and immunohistochemical staining. In the presence of high dose *C. acnes* bacterium the barrier properties deteriorated: TEER and cell index (CI) values gradually decreased, while parallel to that LY penetration increased at 24 and 72 hours after bacterial treatment in the HPV-KER cultures. Dye penetration was also enhanced in the OS models upon *C. acnes* treatment. The level and distribution of TJ proteins also changed, OCLN and ZO-1 increased and CLDN1 decreased after treatment in the monolayer cultures, which was similar to changes we observed in the differentiated, granular layer of the OS models. We hypothesize that *C. acnes* may actively modify the properties of our epidermal barrier by changing the expression and localization of certain TJ proteins and through this it can play a role in the maintenance of cutaneous homeostasis.

P.D4.09.03
A role for miRNA-mRNA interactions in host immunomodulation during controlled human hookworm infection

***M. M. Cooper**, S. Staal, C. Loiseau, T. S. Watkins, M. Field, J. Croese, J. J. Miles, A. Loukas, D. L. Doolan, P. Giacomin;*
Australian Institute of Tropical Health and Medicine, Smithfield, Cairns, Australia.

Helminths have evolved to modulate the human immune response. Recent reports have demonstrated that live helminth therapy has a beneficial effect in treating inflammatory disease, suggesting that helminths alter host immune profiles by promoting an anti-inflammatory environment. MicroRNAs (miRNAs), small negative regulators of mRNA translation, have recently been implicated in immune control in other diseases. We hypothesised that miRNAs play an important role in immumomodulation during helminth infection. Previous investigations of the human immune response to helminths have largely focused on field studies, where significant confounders have influenced study outcomes.
To define the role of miRNA in immunomodulation during helminth infection, we utilised a unique, well-controlled model of experimental *Necator americanis* infection in naive individuals and conducted a comprehensive study of host blood miRNA and mRNA expression. We demonstrate for the first time that hookworm infection induces significant changes to host miRNA and mRNA profiles (Principal Components Analysis, $p<5x10^{-5}$ and $p<5x10^{-4}$, respectively) that are readily detectable in peripheral blood. Specifically, we identified a panel of 18 immune and T cell related mRNAs and 29 miRNAs that are significantly associated with helminth infection and with the degree of infection-induced eosinophilia (all $p<5x10^{-2}$). We further identified a network of miRNA-mRNA interactions resulting from helminth infection that is involved in promoting a Th2-biased immune response.
This study expands our understanding of the immune response to parasitic worm infections and has significant implications for the development of helminth-based, immunomodulatory therapies for inflammatory disease.

P.D4.09.04
ADAM10 and ADAM17 control of skin dendritic cell function

***N. Diener**[1], R. Backer[1], S. Papaioannou[1], K. Dietze-Schwonberg[2], E. von Stebut[2,3], B. E. Clausen[1];*
[1]Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-University Mainz, 55131 Mainz, Germany, [2]Department of Dermatology, University Medical Center of the Johannes Gutenberg-University Mainz, 55131 Mainz, Germany, [3]Department of Dermatology, University of Cologne, 50937 Cologne, Germany.

Dendritic cells (DC) are strategically positioned at epithelial borders to the environment like the skin and are crucial modulators of immune responses. The 'a disintegrin and metalloproteinase' (ADAM) family of surface-expressed ectodomain-shedding proteases regulate multiple cell functions such as cell adhesion, migration or cytokine release/ signaling. Dysregulated shedding by ADAM10 and ADAM17 is critical for the development of different immune-mediated diseases. Both sheddases are expressed on DC in the skin and skin-draining lymph nodes (sdLN). To investigate their role in DC, we generated conditional knockout mice of either one of these proteases in all CD11c$^+$ cells ($ADAM10^{\Delta CD11c}$ and $ADAM17^{\Delta CD11c}$ mice).
Analysis by flow cytometry revealed reduced DC numbers in the skin of $ADAM10^{\Delta CD11c}$ mice, whereas DC homeostasis in $ADAM17^{\Delta CD11c}$ mice was unchanged compared to control mice. Although DC migration to the sdLN was not altered under steady-state conditions and following FITC-painting in $ADAM10^{\Delta CD11c}$ and $ADAM17^{\Delta CD11c}$ mice *in vivo*, it was significantly decreased in skin explant cultures *in vitro*. Moreover, while the phenotypic maturation of bone marrow-derived DC (BMDC) was only impaired in the absence of ADAM17, both ADAM10- and ADAM17-deficient BMDC secreted reduced levels of TNF-α upon TLR stimulation. Intriguingly, after physiologic low-dose infection with *Leishmania major*, $ADAM17^{\Delta CD11c}$ mice developed significantly larger, persisting skin lesions with increased parasite burdens as compared to controls with self-healing lesions. In conclusion, these data demonstrate that ADAM10 and ADAM17 regulate DC homeostasis and maturation/activation. In ongoing experiments we are dissecting the mechanism of ADAM17 governing skin DC function during cutaneous leishmaniasis.

P.D4.09.05
Characterisation of *Schistosoma mansoni* Larval Extracellular Vesicle protein 1 (SmLEV1) an immunogenic, schistosome-specific protein, exhibiting developmentally regulated alternate splicing

***T. A. Gasan**[1], S. Wilson[2], J. Wawrzyniak[2], E. M. Tukahebwa[3], K. F. Hoffmann[1], I. W. Chalmers[1];*
[1]IBERS, Aberystwyth, United Kingdom, [2]Cambridge University, Cambridge, United Kingdom, [3]Ugandan Ministry of Health, Kampala, Uganda.

An integral component of cellular communication, Extracellular Vesicles (EV) have been described in protozoa and metazoan parasites. Both larval and adult *Schistosoma mansoni* worms release pre-packaged EVs, but to what end? Characterising proteins within schistosome EVs will aid in discerning their function(s) and may help develop schistosomiasis control strategies. Threfore, this project aims to characterise the most abundant EV protein in the tissue-migrating schistosomula stage - *Schistosoma mansoni* Larval Extracellular Vesicle protein (SmLEV1). Comparative sequence analysis demonstrates that SmLEV1 has orthologs in all published *Schistosoma* genomes, but not outside of the genus, nor has any characterised protein domains. By employing qRTPCR, we discovered differential expression of SmLEV1 across the schistosome lifecycle, with peak expression in cercariae as well as male-biased expression in sexually-reproductive adults. Importantly, SmLEV1 exhibits developmentally regulated alternative splicing during infection of the mammalian host. Cercariae displayed a significantly different population of isoforms, with over twice the level of exon-5 expression, compared with adult worms, but only two-thirds the expression of exon-8. Recombinant expression of SmLEV1, has enabled investigation of the host's response to SmLEV1, in the mouse model and endemic human populations.

Interestingly, preliminary serological analysis from *S. mansoni* infected individuals shows a strong IgG_1 response against SmLEV1 with minimal antigen-specific IgG_4 and IgE; this finding is congruent to antibody responses generated against other surface/secreted schistosome proteins. Collectively, these results highlight SmLEV1 as an abundant, novel schistosome-specific, EV protein. Finally a mouse vaccination trial has been conducted to investigate the potential protective capabilities of an SmLEV1 vaccine.

P.D4.09.06
Co-inhibitory receptors expression on $CD8^+$ T cells during *T. cruzi* infection

***R. Grote-Gálvez**, Y. Arana, T. Jacobs;*
Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

Trypanosoma cruzi is an obligate intracellular protozoan parasite that in 30 % of cases causes Chagas disease. Chagas disease remains the most important neglected vector-borne disease in Latin America affecting more than 10 Million people. Although an initial $CD8^+$ T cell mediated immune response controls parasite replication successfully, a complete clearance fails, which leads to chronic infection. To address this question, we established mouse-*models* based on infection of C57BL/6 mice with different *T. cruzi* strains. We found that during acute infection with *T. cruzi* the T cell compartment is modulated by transient induction of different co-inhibitory molecules. Tim-3 was most significantly induced and this upregulation correlated with a reduced TNFα production. Using different blocking strategies, we explored Tim-3 function during infection *in vivo*. The blockade of Tim-3 restored $CD8^+$ T cell function and reduced parasitic reservoirs in the tissue. We also found that Tim-3 ligands were strongly expressed on cells from the myeloid compartment. According to our data, we think that the upregulation of co-inhibitory receptors, in context of acute infection, might be a physiological mechanism to avoid immunopathology but *T. cruzi* exploits these pathways to alter parasite specific $CD8^+$ T cell responses and circumvent elimination. We hypothesize that, this provides parasites with valuable time to disseminate throughout the host and to hide in multiple tissues. Our results are an important contribution towards the better understanding of the immune evasion strategies of *T. cruzi* and identification of potential targets for novel immunotherapies.

P.D4.09.07
Helminth-induced interference with bystander immune responses and the role of type 1 regulatory T cells

***W. Hartmann**, M. Brunn, M. Breloer;*
Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

Helminths are large multicellular parasites that infect approximately one third of the human population. To prolong their survival, helminths manipulate the immune response of their hosts. Thereby, not only helminth-specific but also non-helminth-specific bystander immune responses such as the immune response to a vaccine are suppressed. We have previously shown that infection of mice with the filarial nematode *Litomosoides sigmodontis* leads to systemic suppression of IgG responses to thymus-dependent model antigens. This suppressive status was still visible after clearance of *L. sigmodontis* infection indicating that immunosuppression, once established, is preserved independent of the presence of living helminth parasites. We now demonstrate the relevance of this helminth-induced interference with bystander immune responses and performed vaccinations with the commercially available anti-influenza vaccine*:* Reduced hemagglutinin-specific Ig responses were linked with an impaired protection against influenza H1N1 A/Hamburg/NY1580/09 challenge infection. Mechanistically, B cell function was suppressed indirectly, via accessory follicular T helper cells (TFH). Likewise proliferation of adoptively transferred ovalbumin-specific CD4+ T cells was suppressed in *L. sigmodontis*-infected mice, reiterating suppressed TFH expansion. To analyse the mechanism we performed flow cytometry cluster analysis. $Foxp3^+$ regulatory T cells increased locally, but not systemically and were dispensable for helminth-induced suppression of bystander immune responses. By contrast, we observed a sustained local and systemic expansion of type 1 regulatory T cells expressing LAG-3 and CD49b in helminth-infected mice and those with a history of helminth infection. We are currently characterizing the role of Tr1 cells as potential mediators of suppression.

P.D4.09.08
Mycobacterial Growth Inhibition is associated with trained innate immunity

***S. A. Joosten**[1], K. E. van Meijgaarden[1], S. M. Arend[1], C. Prins[1], F. Oftung[2], G. Korsvold[2], S. V. Kik[3], R. J. Arts[4], R. van Crevel[4], M. G. Netea[4], T. H. Ottenhoff[1];*
[1]Leiden University Medical Center, Leiden, Netherlands, [2]Norwegian Institute of Public Health, Oslo, Norway, [3]KNCV Tuberculosis Foundation, The Hague, Netherlands, [4]Radboud University Medical Center, Nijmegen, Netherlands.

The lack of defined correlates of protection hampers development of vaccines against tuberculosis (TB). *In vitro* mycobacterial outgrowth assays are thought to better capture the complexity of the human host/ *Mycobacterium tuberculosis* (Mtb) interaction. Here, we used a PBMC-based "mycobacterial-growth-inhibition-assay" (MGIA) to investigate the capacity to control outgrowth of Bacille Calmette-Guérin (BCG). Interestingly, strong control of BCG outgrowth was observed almost exclusively in individuals with recent exposure to Mtb, but not in (long-term) latent TB infection, and only modestly in BCG vaccinees. Mechanistically, control of mycobacterial outgrowth strongly correlated with the presence of a $CD14^{dim}$ monocyte population, but also required the presence of T-cells. The non-classical monocytes produced CXCL10, and CXCR3-receptor blockade inhibited the capacity to control BCG outgrowth. Expression of CXCR3 splice variants was altered in recently Mtb exposed individuals. Since we observed strong MGIA control recently after Mtb exposure and we found a strong association with monocytic cells we hypothesized that trained innate immunity was responsible for the observed MGIA control. Indeed, cytokines previously associated with trained immunity were detected in MGIA supernatants, and CXCL9, CXCL10 and CXCL11 represent new markers of trained immunity. These data indicate that CXCR3-ligands are associated with trained immunity and critical factors in controlling mycobacterial outgrowth. In conclusion, control of mycobacterial outgrowth early after exposure to Mtb is the result of trained immunity mediated by a CXCL10-producing non-classical $CD14^{dim}$ monocyte subset.

P.D4.09.09
Enhanced cancer immunosurveillance by viral infection and Toll-like receptor ligation

***M. F. Mandour**[1,2], J. P. Coutelier[1];*
[1]Deduve institute - UCL, Brussels, Belgium, [2]Department of Clinical Pathology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.

Introduction: At early stages of cancer development, appropriate immunosurveillance eliminates most of the transformed cells. In addition to cytotoxic T cells, innate immune cells such as natural killer cells and NK/T cells play a major role in this protection against cancer development, especially through production of interferon-gamma (IFN- γ). Since infections deeply modulate the immune microenvironment and particularly its innate components, we investigated their role in cancer immunosurveillance. Materials and Methods: The effect of infections on plasmacytoma (TEPC.1033.C2) and mesothelioma (AB1) cell growth was analysed in BALB/c mice after infection with lactate dehydrogenase-elevating virus (LDV), a usually non-pathogenic mouse nidovirus. Infections were also mimicked by ligation of various Toll-like receptors (TLRs). The mechanisms of immunosurveillance were analysed by using anti-NK cell depleting polyclonal antibody and cytokine neutralizing monoclonal antibodies. Results: Acutely infected animals were significantly protected against both plasmocytoma and mesothelioma development. The protection was mediated by NK cell activation, through IFN- γ production. In addition, TLR-3, 7 and 9 ligation significantly protected mice against mesothelioma, but not plasmacytoma development. Conclusions: Our results indicate that modulation of the mouse immune microenvironment, and especially of innate immune responses, following either a non-pathogenic viral infection or a TLR ligation protects against mesothelioma and plasmacytoma early development.

P.D4.09.10
Hypoxia-inducible factor-1 alpha deficiency results in dysregulated lipid metabolism associated with increased susceptibility to*Leishmania donovani*infection

***I. Mesquita**[1,2], C. Ferreira[1,2], D. Moreira[1,2], G. Kluck[1,3,2], A. Barbosa[1,2], E. Torrado[1,2], R. Dinis-Oliveira[4,5,6], F. Rodrigues[1,2], C. Cunha[1,2], A. Carvalho[1,2], A. Castro[1,2], J. Estaquier[7,8], R. Silvestre[1,2];*
[1]Life and Health Sciences Research Institute (ICVS), School of Medicine, Braga, Portugal, [2]ICVS/3B's–PT Government Associate Laboratory, Braga, Portugal, [3]Medical Biochemistry Institute, Rio de Janeiro, Brazil, [4]Faculty of Medicine, Porto, Portugal, [5]University Institute of Health Sciences, Porto, Portugal, [6]Faculty of Pharmacy, Porto, Portugal, [7]Université Paris Descartes, Paris, France, [8]Centre de Recherche du CHU de Québec, Université Laval, Québec, Canada.

Metabolic manipulation of host cells by intracellular pathogens is recognized to play an important role in the pathology of infection. Hypoxia inducible factor-1 alpha (HIF-1α) is a critical regulator of myeloid cell function, namely in infectious contexts. We demonstrate that specific deletion of HIF-1α in the myeloid compartment increases susceptibility to *Leishmania donovani* infection, which associates with an increased de novo lipogenesis. Infected myeloid-restricted HIF-1α-deficient mice developed hypertriglyceridemia and enhanced accumulation of lipids in splenic myeloid cells. Upon pharmacological inhibition of fatty acid synthase, the observed susceptibility is abrogated, and the parasite load is restored to the levels of WT counterparts. Importantly, *Leishmania* infection of macrophages from individuals with homozygous loss-of-function polymorphisms in HIF1A gene displayed increased parasite burdens associated with lipid accumulation. Our results provide direct evidence that genetic deficiency of HIF-1α impacts the anti-*Leishmania* effect of the myeloid compartment via lipid accumulation.

P.D4.09.11

NLRP10 affects the stability of Abin-1 to control inflammatory responses

N. Mirza[1], *A. Sowa*[1], *K. Lautz*[2], *T. Kufer*[1,2];
[1]*Institute of Nutritional Medicine, University of Hohenheim, 70599 Stuttgart, Germany,* [2]*Institute for Medical Microbiology, University of Cologne, 50935 Cologne, Germany.*

Introduction: NOD-like receptors (NLR) are critical regulators of innate immune signaling with a conserved structure containing a central oligomerization NACHT domain, an N-terminal interaction domain and a variable number of C-terminal leucine rich repeats (LRRs). NLRP10 is the only NLR-protein lacking the LRR and has been implicated in multiple immune pathways, including the regulation of inflammatory response towards *L. major* and *S. flexneri* infection. However, our current understanding of NLRP10 is limited and its function in immune signaling pathways require further clarification.
Materials and Methods: Interaction of NLRP10 and Aibn-1 was identified by a yeast to hybrid screening and verified in co-immunoprecipitation experiments. *Shigella flexneri* was used as an infection model in human epithelial cell lines and primary murine cells derived from *Nlrp10* knock-out animals.
Results: Here we identify Abin-1, a negative regulator of NFκB, as an interaction partner of NLRP10, which binds to the NACHT domain of NLRP10. While the interaction of the proteins increased with time of *S. flexneri* infection, NLRP10 decreased Abin-1 protein stability in response to bacterial infection in HEK cells as well as in murine embryonic fibroblasts, primary murine skin fibroblasts and keratinocytes derived from *Nlrp10* knockout mice.
Conclusions: We revealed a novel interaction between NLRP10 and Abin-1 and a subsequent regulation of Abin-1 protein levels by NLRP10. Our data give insight into the molecular mechanism underlying the function of NLRP10 in innate immune responses and provide an explanation for the role of NLRP10 in bacterial induced inflammatory responses.

P.D4.09.12

Quantitative proteomic analysis of CD4+T cells-expressing HCV core protein

C. Fernández Ponce[1], *M. C. Durán-Ruiz*[1], *J. Olid Franco*[1], ***L. Olvera-Collantes***[1], *A. Rodríguez-Moreno*[1], *J. Niño-Ramirez*[1], *J. P. Muñoz- Miranda*[1], *M. M. Arbulo-Echevarria*[1], *I. Narbona-Sánchez*[1], *A. Serna-Sanz*[2], *C. Baumann*[2], *R. Litrán*[3], *E. Aguado*[1], *F. García-Cózar*[1];
[1]*Department of Biomedicine, Biotechnology and Public Health, University of Cadiz - Institute of Biomedical Research Cadiz (INIBICA), Cadiz, Spain,* [2]*Sciex, Darmstadt, Germany,* [3]*Department of Condensed Matter Physics, University of Cádiz, Puerto Real, Spain., Cadiz, Spain.*

Hepatitis C virus (HCV) infection is a significant public health problem affecting more than 80 million people worldwide. Up to 80% of HCV infected patients develop chronic viral hepatitis with clinical progression to hepatic fibrosis, cirrhosis, liver failure and hepatocellular carcinoma. Currently, the molecular mechanisms used by HCV to cause a chronic infection and their implications in the innate and adaptive immune system have not been fully explained. Several HCV viral proteins have been described as immune modulators, being HCV core protein closely related with pathogenicity, virulence, immune evasion and immune regulation. Interestingly, the presence of HCV Core protein in $CD4^+$ T cells has been associated with the induction of a regulatory T cell phenotype. In order to elucidate the molecular networks changes induced by the presence of HCV Core protein in $CD4^+$ T cells, we performed a quantitative analysis of the proteome of T lymphocytes transduced with Core protein. The study was accomplished using mass spectrometry. The identified and quantified proteins, and the biological networks associated were analyzed using IPA (Ingenuity pathway analysis) software. We found changes in the expression of proteins implicated in molecular networks related with lymphocyte activation, regulation and reorganization of the cytoskeleton, induction of regulatory activity in T lymphocytes, cell adhesion, tissue invasion and oxidative stress signaling pathways.

P.D4.09.13

Intracellular immunodynamics of lentiviral gene delivery in human Natural Killer cells

C. Pamukcu[1,2], *E. C. Sayitoğlu*[3], *D. Ozkazanc*[1,2], *A. Parlar*[1,2], *A. E. Eyupoglu*[1,2], *B. Erman*[1,2], *A. D. Duru*[3], *T. Sutlu*[1];
[1]*Nanotechnology Research and Application Center, Sabancı University, Istanbul, Turkey,* [2]*Faculty of Engineering and Natural Sciences, Sabancı University, Istanbul, Turkey,* [3]*NSU Cell Therapy Institute, Nova Southeastern University, Fort Lauderdale, United States.*

Cancer immunotherapy approaches using genetically modified NK cells continue to inspire clinical trials with promising results but the protocols for genetic modification of NK cells are suboptimal. NK cells they show strikingly high resistance to lentiviral gene delivery when compared to other cells of the immune system. Previously, we have shown that the use of BX795, a small molecule inhibitor of TBK1/IKKε complex downstream of Toll-like Receptors and RIG-I-like receptors, significantly enhances lentiviral gene delivery to NK cells.
In the current study, we demonstrate that while viral vector entry to NK cells can take place without major problems, the activation of antiviral signaling pathways leads to the intracellular elimination of the vector. To study roles of 20 candidate genes in this process, CRISPR/Cas9 system was used and single-gene disruptions were applied in 293FT and NK-92 cell lines.
Our results demonstrate that capsid recognition by TRIM5α in 293FT cells and dsRNA-induced signaling through RIG-I and TRIM25 in NK-92 cells represent major players affecting lentiviral gene delivery. Additionally, viral vector exposure was shown to increase MAPK activity in host cells, specifically p38 and JNK phosphorylation in NK-92 cells, as observed in wildtype HIV infections.
Overall, this study confirms that lentiviral gene delivery evokes an innate immune response in NK cells through multiple PRRs and cellular restriction factors. Small molecule inhibitors help to overcome this obstacle for promising applications in immunotherapy using genetically modified NK cells.

P.D4.09.14

LPCAT2 Knockdown Influences both Rough and Smooth Lipopolysaccharide-Induced Toll-Like Receptor 4 Signalling in RAW264.7 Macrophages.

V. I. Poloamina*, W. A. Woldie, G. Fejer, S. K. Jackson;*
School of Biomedical Sciences, Faculty of Medicine and Dentistry, University of Plymouth, Plymouth, UK. PL6 8BU, Plymouth, United Kingdom.

Recognition of bacterial lipopolysaccharide (LPS) by TLR4 and its co-receptors leads to inflammation through the interaction of TLR4 signaling adaptor proteins and other proteins downstream. We have previously shown that TLR4 signaling is reduced when LPCAT2 (a phospholipid modifying enzyme) is knocked down. Smooth and Rough LPS may possess differential mechanisms of regulating TLR4 signaling, however, it is not known if the knockdown of LPCAT2 would have the same effect on TLR4 signaling induced by both smooth LPS and rough LPS.
RAW264.7 macrophages were transfected with LPCAT2 siRNA. After 48hours, RAW264.7 were stimulated with 100ng/ml of E. Coli O111: B4, J5, and K12 LPS for 6hours to determine mRNA and TNF-alpha protein, and 24hours to determine IL6 and IP10 protein by ELISA. Total RNA was isolated, quantified, and used for analysis of mRNA expression by real-time RT-PCR. Statistical analysis was carried out using GraphPad Prism Software.
Rough LPS (J5 and K12) induced MyD88-dependent cytokines (IL6 and TNF-alpha) about 2-fold more than smooth LPS (O111:B4), but there was no difference in the induction of MyD88-independent cytokines (IP10 and IFN-beta). Moreover, both rough and smooth LPS-induced cytokines were significantly reduced when LPCAT2 was knocked down (>70 percent).
LPCAT2 does not show a differential influence on inflammation induced by rough and smooth LPS. Moreover, LPCAT2 knockdown might be affecting the MyD88-dependent TLR4 signaling pathway more than the MyD88-independent pathway. LPCAT2 plays a role in TLR4 signaling which is important in innate immunity. Therefore, LPCAT2 can be an effective target for anti-inflammatory treatments.

P.D4.09.15

Evaluation of miRNAs role in the immunopathogenesis of microcephaly caused by ZIKV in experimental models

C. M. Polonio[1], *W. Brandão*[1], *F. Cugola*[2], *N. Zanluqui*[1], *L. Oliveira*[1], *C. Longo*[1], *P. Beltrão-Braga*[2], *J. Peron*[1];
[1]*Neuroimmune Interactions Laboratory - Department of Immunology - University of São Paulo, São Paulo, Brazil,* [2]*Disease Modeling Laboratory- Department of Microbiology - University of São Paulo, São Paulo, Brazil.*

Viral infections have always been the cause of serious human diseases, usually increasing rates of morbidity and mortality worldwide. Recently, the flavivirus Zika virus (ZIKV) was introduced especially in Brazil, causing alarming increase in the number of babies born with microcephaly. The expression of Interferons stimulated genes (ISGs) is very important in blocking viral replication during disease, and they may be modulated by several different factors through post-transcriptional mechanisms, in which, *miRNAs* play a key role. Still very little is known about the involvement of *miRNAs* during ZIKV infection. In this context, we evaluated the role of *miRNAs* during ZIKV experimental infection. We performed miRNAs analysis *in vitro* using cells of the central nervous system and, *in vivo*, with SJL animals susceptible to infection. Analyzing human neuronal precursor cells we observed that miR-29, miR-425, miR-15A and miR-126 were up-regulated. miR-29 is known to be present in HPV patients blood and to induce the PRRSV viral replication. ZIKV was also able to down-regulate 19 different miRNAs, among them, miR-9, known to increase mesenchymal stem cells differentiation towards neuronal cells, being an important factor for neurogenesis. In SJL fetal brain, we observed nine miRNA up-regulated and only miR-32 was down-regulated. Taken together, these data are indicative that ZIKV is able to modulate miRNA profile, evidencing the importance of these regulatory molecules, which may help us to better understand immunopathogenic mechanisms of microcephaly caused by ZIKV.

P.D4.09.16
Site-specific effects of IL-33 treatment during helminth infection

***M. Reitz**, N. Rüdiger, M. Brunn, M. Breloer;*
Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

Strongyloides ratti is a rodent specific parasitic nematode that displays tissue migrating and intestinal life stages. Infective larvae actively penetrate the skin of their host, migrate within 2 days via the skin and lung to the mouth. They are swallowed, reach the intestine and moult to adults that reproduce by day 5. Infected mice terminate the infection in the context of a type II immune response. Thereby infection-induced expression of the alarmin IL-33 by alveolar epithelial cells was shown to promote the type II response in the lung and was associated with efficient expulsion of *S. venezuelensis* from the intestine. Here, we intend to dissect IL-33 mediated effects on the immune response to *Strongyloides* infection in the tissue and the small intestine. *S. ratti* infected mice showed a drastic reduction of parasitic adults in the intestine on day 6 after previous intranasal and intraperitoneal treatment with recombinant IL-33 (rIL-33). The reduced parasite burden correlated with increased activation of mast cells that are central for expulsion of *S. ratti* from the intestine. Of note, also rIL-33 administration after the tissue migration phase reduced intestinal parasite burden. In contrast, numbers of migrating larvae in skin, lung and head were significantly increased in rIL-33 treated mice indicating that the reduced intestinal parasite burden is not due to an improved immunity in the tissue. In summary, our data show that IL-33 displays contradictory and site specific effects on tissue migrating and intestinal *S. ratti* parasites. We are currently investigating the underlying mechanism.

P.D4.09.17
Malaria-induced FOXP3$^-$ regulatory T cells in disease progression and memory establishment

***M. Riehn**, M. S. Mackroth, A. Abel, C. Steeg, T. Jacobs;*
Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

Malaria is still a major burden on global health with approximately 1200 deaths per day. After infection, immunity has to be strictly regulated to control the parasite but also to avoid immune pathology. Interestingly, in malaria experienced patients protection against severe malaria develops faster than protection against parasitemia.
Recently, we could show that acute malaria induces CD4$^+$PD1$^+$Foxp3$^-$ T cells in humans. These cells can suppress effector T cells, which may contribute to reduced immune pathology but also may suppress protective immunity.
We could confirm induction of these suppressive cells by blood stage infection employing the *P. berghei* ANKA mouse model. High numbers of these T_{r1}-like cells correlated with reduced immune pathology during reinfection, whereas parasitemia is not altered in comparison to the initial infection.
By combining flow cytometry and t-Distributed Stochastic Neighbor Embedding (t-SNE), we could delineate a unique phenotype of these malaria-induced T cells characterized by a high expression of multiple co-inhibitory molecules. The highest amount of these T_{r1}-like cells was found in the liver compared to spleen. Furthermore these cells exhibit different phenotypes in liver and spleen with regard to the expression of co-inhibitory molecules. This indicates an organ-specific component implicated in the induction of malaria-specific T_{r1}-like cells.
Taken together, we could show that malaria-induced T_{r1} cells have an important immune regulatory function in mice and humans and should be considered in malaria therapy and vaccination. Moreover, the accumulation of T_{r1} in the liver highlights the unique tolerogenic environment of this organ and the influence on disease progression.

P.D4.09.18
Batf3 deficient mice are protected against experimental cerebral malaria due to successful immune regulation

*M. P. Borsche[1], J. M. Kuepper[1], P. J. Korir[1], A. Mueller[2], A. Hoerauf[3], K. Hildner[3], I. R. Dunay[4], **B. Schumak**[1];*
[1]Institute of Medical Microbiology, Immunology and Parasitology, University of Bonn, Bonn, Germany, [2]Centre for Infectious Diseases, Parasitology Unit, Heidelberg, Germany, [3]Medical Department 1, University Hospital Erlangen, Erlangen, Germany, [4]Institute of Inflammation and Neurodegeneration, University of Magdeburg, Magdeburg, Germany.

Excessive inflammatory immune responses are made responsible for severe complications during infections with *Plasmodium* parasites such as cerebral malaria (CM). The parasite-specific immune response is initiated by dendritic cells, especially by cross-presenting DCs that efficiently activate cytolytic CD8 T cells. Batf3 ko lack CD8a positive dendritic cells (DCs), which are well known for the induction of strong Th1 responses. Here we show that BATF3 mice were protected from ECM upon infection with *Plasmodium berghei ANKA* (PbA), characterized by a stable blood brain barrier and lacking infiltration of peripheral immune cells into the brain. Importantly, the absence of ECM correlated with attenuated responses of cytotoxic T cells, since their parasite-specific lytic activity as well as production of interferon gamma was strongly decreased and granzyme B was absent. Remarkably, we detected in the ECM-negative mice elevated levels of IL-10 that was produced predominantly by myeloid cells. We conclude that in the absence of excessive Th1 signaling due to the lack of crosspresenting DCs, immune regulatory mechanisms were successfully induced that were associated with protection from excessive brain inflammation.
Funding: BONFOR (JMK, BS), DAAD (PJK) EXC1023 (AH, BS)

P.D4.09.19
Identification of new natural products with anti-microbial activity against Apicomplexa and multiresistant gram-negative rods (4MRGN)

***S. Shaneh Sazzadeh**[1], S. Schmidt[1], K. Buchholz[1], P. Proksch[2], K. Pfeffer[1];*
[1]Institute of Medical Microbiology and Hospitalhygiene, Duesseldorf, Germany, [2]Institute of Pharmaceutical Biology and Biotechnology, Duesseldorf, Germany.

Introduction: Anti-microbial therapies have successfully treated infectious diseases. However, the recent occurrence of (multi-) resistant pathogens increases lethality and morbidity of infected patients. Apicomplexa also develop resistance against established treatments. Therefore the need for new anti-microbial drugs is urgent. The primary aim of this project will be the identification of novel natural products with anti-microbial activities against *Toxoplasma gondii* and multidrug resistant gram-negative rod-shaped bacteria (4MRGN), and the elucidation of their targets in pathogens in order to develop new leads for anti-microbial therapies.
Materials and Methods: Screening of natural products, which are able to inhibit *T. gondii* proliferation without being cytotoxic against HFF (human foreskin fibroblasts). This is accomplished via Toxoplasma proliferation and MTT assays. To identify anti-4MRGN products microdilution assays are performed.
Results: Within a first round of screening, promising candidates could be detected. This project will hopefully identify new anti-microbial products for novel therapies of multiresistant pathogens. Furthermore, after performing the MTT assay none of the natural products demonstrate cytotoxicity against both cell lines, which were used in toxoplasma proliferation assay except Bionectriamide A.
Conclusions: A total of 300 new natural products and 30 derivatives thereof were analysed against *T.gondii* (type I, BK strain and also type II, ME49 strain). Eleven products demonstrated anti-Toxoplasma activity and have been selected for further analyses.
Funding: GRK 2158, DFG

P.D4.09.20
Lymphotoxin β receptor: A crucial role in *Toxoplasma gondii* infection

***A. Wichert**, U. R. Sorg, D. Degrandi, K. Pfeffer;*
Institute of Medical Microbiology and Hospital Hygiene, Düsseldorf, Germany.

Introduction: After infection with the obligate intracellular protozoan parasite *Toxoplasma gondii* (*T. gondii*) the production of cytokines induces potent cell autonomous effector mechanisms which can inactivate the pathogen. Lymphotoxin β receptor (LTβR) signalling plays an important role in efficient initiation of innate and adaptive host responses to a variety of pathogens. The up-regulation of the murine 65kDa guanylate-binding proteins (mGBPs) via interferon γ receptor (IFNGR) signalling plays an essential role in survival of mice after infection with *T. gondii.* mGBPs recruit towards the parasitophorous vacuole (PV) encapsulating the parasite, leading to the disruption of the PV and subsequent killing of the parasite. Compared to wildtype mice, LTβR deficient (LTβR$^-$) mice show a markedly increased mortality and delayed up-regulation of mGBP expression in the acute phase of *T. gondii* infection. Methods: Immune responses (particularly B and T cell responses) as well as mGBP localization and function in LTβR$^{-/-}$ compared to WT mice after *T. gondii* infection were investigated in *in vivo* and *in vitro* experiments. Results: Analysis of IgM and IgG antibody responses suggests defects in Ig-class switching in LTβR$^{-/-}$ mice while FACS analysis of immune cell populations demonstrates that these mice are generally able to generate *T. gondii* specific CD8$^+$ T cells. Initial *in vitro* experiments demonstrate that after IFNγ stimulation mGBPs in LTβR$^{-/-}$ fibroblasts are able to localize to the PV. Conclusion: These data suggest that defects in IFNγ mediated mGBP upregulation as well as dysfunctional Ig-class switching may contribute to the decreased survival rates of LTβR$^{-/-}$ mice.

P.D4.09.21
Host-Targeted Therapeutic Immune Protection against Anthrax

***M. Zeng**[1], Y. Yan[1,2], H. Wang[1,2], Y. Chen[1], Z. Zheng[1,2], H. Yang[1,2], S. Franco[1];*
[1]Center of Emphasis in Infectious Diseases, Texas Tech University Health Sciences Center El Paso, El Paso, United States, [2]Key Laboratory of Oral Medicine, Guangzhou Institute of Oral Disease, Stomatological Hospital of Guangzhou Medical University, Guangzhou, China.

Introduction: To combat emerging and re-emerging infectious disease, it is more cost effective to use therapeutic vaccines for postexposure treatment than mass vaccination with preventive vaccines. Anthrax is an infectious disease caused by *Bacillus anthracis* that can secrete anthrax toxins including protective antigen (PA), lethal factor (LF), and edema factor (EF). Previously, we have shown that RNA inhibition of anthrax toxin receptors (TEM8 and CMG2) was protective against the cytotoxicity of anthrax toxins. However, inefficient cytosolic delivery and toxicity of siRNA delivery vehicles limit the use of siRNA as therapeutics.

Materials and Methods: In this study, we have developed a detoxified anthrax edema toxin, which consists of PA and nontoxic N-terminal fragment of EF (EFn) conjugated with a peptide nona-D-arginine residues (EFn-9dR) to enable siRNA binding. The detoxified toxin-siRNA was used to treat cells and C57BL/6 mice and evaluate if they can be protected against anthrax.
Results: The detoxified toxin complex was able to deliver specific siRNA to induce *cmg2* gene silencing in different cell lines and C57BL/6 mice, and provide significant protection against anthrax lethal toxin challenge. Survived mice from toxin challenge were fully protected against lethal challenge with *B. anthracis* Sterne spores. The immune protective mechanism is mainly due to the significantly high serum neutralizing antibody response against anthrax toxins in these mice.
Conclusions: The detoxified anthrax toxin complex provides a tool for delivery of host-targeted siRNA into anthrax pathogenesis-associated host cells, and it can be used as a lifesaving postexposure therapeutic vaccine against anthrax.

P.D4.10 Exploiting host pathogen interaction - Part 10

P.D4.10.01
Influence of regulatory T cells in immune answer of dogs with visceral leishmaniasis

***P. H. L. Bertolo**, P. R. Moreira, M. B. Conceição, R. O. Vasconcelos;*
Sao Paulo State University (UNESP), Jaboticabal, Sao Paulo, Brazil.

Introduction - The visceral leishmaniasis (VL) is a chronic zoonotic disease, marked by macrophages infection with *Leishmania infantum,* which is distributed to many organs. Parasitic load is very different in each organ, showing that some tissues are more susceptible to this parasite, as spleen. The regulatory T cells (Treg) can avoid pro inflammatory answer, thus, evaluate your role in canine VL would be important. This way, the aim of this study was detect Treg cells in the popliteal and pre-scapular lymph node, liver, spleen and skin (nose and ear) of dogs naturally infected with *L. infantum*, in endemic area for VL. Methods - The dogs were distributed in two groups: infected (n=29) and control (n=5), this had dogs of free VL areas. Immunohistochemistry was used to detect Treg cells in the tissue (FoxP3 antibody, diluted in 1:2500). Results - The immunostaining of Treg cells was observed predominantly at cell nucleon. In lymph nodes it was seen more at cortical area, in liver at intralobular granuloma, in spleen, at white pulp and in skin, at granulomas present around the cutaneous appendages. Comparing these two groups by each organ, significant difference was observed for Treg cells load in nose (P=0,0118), spleen (P=0,0541) and pre-scapular lymph node (P=0,0214), much of these cells was seen in infected group. Conclusion - Lymphoid organs and skin maybe has some influence in Treg cells regarding parasite surviving.
Keywords: Parasiste immunology, Regulatory cells, Veterinary immunology, Lymphoid organs.
Financial support: Fapesp (procedural number 2013/00763-4).

P.D4.10.02
Vitamin D deficiency:a potential predisposition factor for sepsis development

***M. Buc**[1], M. Olejarova[1], A. Dobisova[2], J. Koutun[2], S. Blazickova[3], M. Bucova[1];*
[1]Institute of Immunology, Bratislava, Slovakia, [2]1st Department of Anaesthesiology and Intensive Care Medicine, Comenius University Faculty of Medicine and University Hospital, Bratislava, Slovakia, [3]Piestany Laboratories, Ltd, Piestany, Slovakia.

Introduction: Vitamin D hormonal actions influence mineral metabolism and skeletal health. However, vitamin D has an impact on function of the immune system, e.g. it acts as important stimulant for innate immunity and enhances the antimicrobial effects of macrophages and monocytes. In the frame of our project to find biomarkers distinguishing sepsis from non-infectious SIRS, we paid attention to this hormone. Material and methods: We investigated 32 patients suffering from SIRS/sepsis. 5 ml of blood was taken into EDTA tubes on day of admission to the clinic and subsequently on days 2, 3, 5, 7 or exceptionally day 10. Different cytokines and inflammatory markers were investigated. Except them, plasma levels of 25hydroxyvitamin D /25(OH)D/ were evaluated by electrochemiluminiscent binding test and were correlated with levels of CRP and presepsin (sCD14) - all from the 1st sample. Results: We found significantly decreased levels of 25(OH)D in septic patients (N=25; 11,084±4,965 µg/l) compared to SIRS patients (N=7; 19.071±8.44 µg/l; p=0.0097). The significant lower levels of 25(OH)D were found also in the group of patients who did not survive (N=5; 6.540 ±3.966) compared to those, who survived the 7th day of hospital care (N=27; 13.996±6.416; p=0.0076). We also disclosed a correlation between the levels of 25(OH)D, CRP (p=0.0003), presepsin (p=0.0032), and SOFA (sequential organ failure assessment) score (p=0.0385). Conclusions: Our results indicate that low levels of vitamin D predispose patients to the development of sepsis and influence their survival.

P.D4.10.03
Depletion of regulatory T cells in ongoing Paracoccidioidomycosis reverses disease severity

*N. Galdino, F. V. Loures, E. F. Araujo, T. A. Costa, **V. L. G. Calich**;*
Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil.

In many infectious diseases, the suppressive activity of Treg cells has been associated with deleterious effects, however in certain experimental settings this activity can be protective due to the control of excessive inflammation. In paracoccidioidomycosis, the most important deep mycosis in Latin America, most studies on Treg cells function were performed by depleting Treg cells before or early in the infection. However, human PCM is diagnosed late, when the disease is already established. This fact led us study the effect of Treg cells depletion in a model of ongoing pulmonary PCM. Then, Treg cells depletion was performed by treatment of C57BL/6DTR/eGFP (DEREG) mice with diphtheria toxin (DT) after 3 weeks of infection with 1 x 10^6 *Paracoccidioides brasiliensis* yeasts by the intratracheal route. At weeks 6 and 10 after infection, DT treated DEREG mice showed reduced number of Treg cells that was associated with decreased fungal burdens in the lungs, liver and spleen as well as diminished tissue pathology when compared with control mice (infected and treated with saline). Additionally, DT treated mice showed an increased influx of CD4+ and CD8+ effector cells into the lungs paralleling the increased production Th1 and Th17 cytokines and reduced mortality. Altogether, our data demonstrate for the first time the beneficial effects of Treg cells depletion in established PCM. This procedure ameliorated all parameters of disease severity and immunity. More importantly, these findings indicate that the control of Treg cells in the course of PCM can be explored as a novel immunotherapeutic procedure.

P.D4.10.04
Identification of *Borrelia burgdorferi* phagocytic receptors and their role in the inflammatory response

***A. Carreras Gonzalez**, D. Barriales, I. Martin-Ruiz, J. Lavín, M. Azkargorta, N. Navasa, F. Elorza, A. M. Aransay, H. Rodriguez, J. Anguita;*
CIC bioGUNE, Derio, Spain.

Borrelia burgdorferi, the causative agent of Lyme disease, causes a range of inflammatory complications including arthritis, carditis and immune system disorders that can become long lasting if not properly treated.
In the context of carditis, as the heart presents little exposure to antibodies, the control of the infection relies majoritarily on the phagocytic activity of macrophages. In turn, phagocytosis is required for the full response of macrophages including the production of proinflammatory factors. In spite of its importance, little is known about the complement of receptors and signals that mediate the internalization of *B. burgdorferi*.
Through the use of transcriptomic and proteomic approaches we identified potential phagocytic receptors implicated in the clearance of *B. burgdorferi* in human and mice, with the differential capacity to induce pro-or anti-inflammatory signals. As a result, we have found that Fc Receptor CD64 seem to play an important role in the clearance of the bacteria and in the inflammatory outcome. Moreover, we have described the regulatory role of the TLR family member, CD180, which influences *Borrelia burgdorferi* phagocytic receptor CR3, and is implicated in the complex pathogen-host defense equilibrium.
The identification of the full complement of phagocytic receptors and their pro/anti-inflammatory activity will allow to define internalization alternatives and, in the future, devise novel strategies to increase phagocytosis without a consequent intensification of local inflammatory responses.

P.D4.10.05

Low CCR5 expression protects specific CD4+ T cells of HIV controllers from viral entry

M. Claireaux[1,2], R. Robinot[1,2], I. Staropoli[1,2], A. Brelot[1,2], M. Héry[1,2], M. Patgaonkar[1,2], A. Nouël[1,2], S. Volant[3], E. Perthame[3], F. Boufassa[4], D. Zucman[5], P. de Truchis[6], O. Lambotte[7,8,9], ***L. A. Chakrabarti***[1,2]*;*

[1]Virus and Immunity Unit, Pasteur Institute, PARIS CEDEX 15, France, [2]INSERM U1108, Paris, France, [3]Bioinformatics and Biostatistics Hub, Pasteur Institute, PARIS CEDEX 15, France, [4]INSERM U1018, Center for Research in Epidemiology and Population Health, Le Kremlin-Bicêtre, France, [5]HIV Unit, Foch Hospital, Suresnes, France, [6]AP-HP, Infectious and Tropical Diseases Department, Raymond Poincaré Hospital, Garches, France, [7]INSERM U1184, Center for Immunology of Viral Infections and Autoimmune Diseases, Le Kremlin-Bicêtre, France, [8]AP-HP, Department of Internal Medicine and Clinical Immunology, University Hospital Paris Sud, Le Kremlin-Bicêtre, France, [9]Université Paris Sud, UMR1184, Le Kremlin-Bicêtre, France.

HIV controllers, who spontaneously contain HIV replication to very low levels, develop particularly efficient antiviral T responses. To gain insights into the contribution of the CD4 helper subset to HIV control, we characterized the differentiation status of HIV-specific CD4+ T cells at the single cell level. CD4+ T cells reactive with MHC-II tetramers specific for the most immunodominant HIV epitope (Gag293) were analyzed by multiplexed real-time qPCR combined with multiparametric flow cytometry. HIV controllers from the ANRS CODEX-CO21 cohort with a viral load <50 copies/mL were compared to efficiently treated patients with an equivalently low viral load. Gag293-specific cells from HIV controllers proved to express lower levels of PD-1 and of the HIV coreceptor CCR5 than those of treated patients, while CCL5 and TRBV2 expression were increased. Interestingly, HIV controller specific cells proved less susceptible to fusion with an HIV-JRFL reporter virus (P=0.017). Moreover, CCR5 expression correlated with HIV fusion (R=0.83, P<0.005). CCR5 expression in total CD4+ T cells did not reveal significant differences between groups. However, a negative correlation was observed between CCR5 expression in total CD4+ T cells and the frequency of Gag293-specific cells, indicating that the subset of controllers with low CCR5 expression maintained strong CD4 responses. Genetic analysis of one controller with particularly low fusion susceptibility uncovered biallelic mutations that impaired CCR5 expression. Taken together, these findings reveal a lower susceptibility of HIV controller specific CD4+ T cells to HIV entry, and point to a role for low CCR5 expression in promoting spontaneous HIV control.

P.D4.10.06

Toll-like receptor 9 is required for the maintenance of $CD25^+FoxP3^+CD4^+T_{reg}$cells during *Listeria monocytogenes*infection

J. S. Dolina*, J. Lee, S. P. Schoenberger;*
La Jolla Institute for Allergy and Immunology, La Jolla, United States.

It has long been appreciated, but not understood, that the $CD8^+$ cytotoxic T lymphocyte (CTL) dependence on $CD4^+$ T cell help (T_h) is conditional; needed for some immunogens but not others. One explanation for this phenomenon envisions T_h requirement as an intrinsic property of the pathogen itself rather than its introduction to the immune system. Here we show that dependence of the optimal $CD8^+$ T cell response to *Listeria monocytogenes* (*Lm*) on $CD4^+$ T cells is a function of the immunogen dose used for priming, with low dose *Lm* (LD; 50 or 10^3 CFU WT or Δ*actA*, respectively) inducing a primary antigen-specific CTL response profoundly dependent on $CD4^+$ T_h cells while that induced by high dose *Lm* (HD; 4×10^3 or 10^6 CFU WT or Δ*actA*, respectively) is significantly inhibited by $CD4^+CD25^+FoxP3^+$ regulatory T cells (T_{reg}). The T_h-independence of HD immunization is not overcome by additional antigen but instead involves the inflammatory response to more bacteria. Evaluation of various toll-like receptor (TLR) pathways as the relevant sensing mechanism showed that HD immunization in the absence of TLR9 results in a simultaneous loss of $CD25^+FoxP3^+CD4^+$ T_{reg} cells and increase in conventional $CD4^+$ T_h cells and CTLs. Our data thus reveal that the CTL response to the same pathogen is determined by distinct roles for $CD4^+$ T cells as helpers versus regulators based on immunogen dose and demonstrate a previously undescribed role for TLR9 in the regulation of $CD4^+$ T_h and T_{reg} cells.

P.D4.10.07

Focussing in on inflammasomes: Exploring new regulators of innate immune signalling pathways in the host defence against *K.pneumoniae*

K. Edwards*[1], A. Dumigan[1], Y. Dombrowski[1], J. Bengoechea[1], A. Kissenpfennig[1], P. Moynagh[1,2];*
[1]Centre for Experimental Medicine, Queen's University Belfast, Belfast, United Kingdom, [2]Institute of Immunology, Department of Biology, National University of Ireland Maynooth, Maynooth, Ireland.

Introduction: The innate immune system induces inflammation in response to injury or infection. Inflammasomes are innate immune signalling complexes vital in the activation of caspase-1, inflammation and wound healing. This study explored the contributions of inflammasomes to host defence against *Klebsiella pneumoniae* (*Kp*), a multi-drug resistant pathogen, and whether *Kp* virulence mechanisms target inflammasome pathways. Materials and Methods: Two *Kp* strains with different virulence, *Kp43816 (Kp43)* and *KP52145 (Kp52)*, were used to infect C57BL/6J mice intranasally for 24h to assess inflammation (ELISA) and virulence within the lung, spleen and NALT (CFU). Bone marrow-derived macrophages (BMDMs) from C57BL/6J and NLRP3 knockout (KO) mice were infected with *Kp43* or hypermucoid *Kp52* at 70:1 multiplicity of infection and assessed for bacterial burden, inflammasome activation (Western blot) and inflammation. Results: *Kp43*-infected C57BL/6J mice had significantly greater weight loss (p=0.0208) and bacterial burden within the nasal-associated lymphoid tissue (NALT) (p<0.0001) and spleen (p=0.0133) compared to *Kp52*-infected mice. *In vitro*, after 24h, *Kp43*-infected BMDMs produced more mature IL-1β (p≤0.01) and caspase-1 than *Kp52*-infected BMDMs. Phagocytosis of *Kp43* by BMDMs was increased compared to *Kp52* (p<0.001). Intracellular survival of either strain of *Kp,* however, did not differ. NLRP3KO BMDMs mounted effective inflammasome and anti-bacterial responses to *Kp*. Conclusions: Our results demonstrate that hypermucoid *Kp52* elicits a weaker inflammatory response and is therefore less virulent compared to *Kp43 in vivo*. NLRP3 was not vital for clearing *Kp* infection suggesting other inflammasomes are responsible. Informatively targeting inflammasomes during infection may be useful in aiding clearance of *Kp* infection.

P.D4.10.08

Transcriptional regulation of CD38 by Liver X receptors and inflammation: roles for CCAAT/enhancer-binding protein β (C/EBPβ)

E. Glaria*[1], J. Matalonga[1], J. Saura[2,3], A. F. Valledor[1];*
[1]University of Barcelona, Barcelona, Spain, [2]University of Barcelona/IDIBAPS, Barcelona, Spain, [3]Institute of Neurosciences, University of Barcelona, Barcelona, Spain.

Macrophages are essential players of the innate immune response against pathogens. Following recognition of pathogen-associated molecular patterns (PAMPs), they produce inflammatory mediators and trigger phagocytosis to fight infection. Liver X Receptors (LXRs) are ligand-activated transcription factors with relevant activities in the regulation of metabolism and the immune response. Recently, our group identified a new role for LXRs reducing macrophage infection by invasive *Salmonella* Typhimurium through transcriptional activation of CD38 and a subsequent increase in its NADase activity. Interestingly, we found that inflammatory mediators and bacterial components act synergistically with LXR agonists to induce CD38 expression. Remarkably, CD38 expression in bone marrow-derived cells was necessary for the ameliorating effects of pharmacological administration of an LXR agonist in an *in vivo* model of infection by *Salmonella*. To explore mechanisms of cooperation between inflammatory stimuli and LXRs, we investigated the role of the transcription factor C/EBPβ in the transcriptional control of CD38. Macrophages were differentiated *in vitro* from bone marrow precursors of wild-type mice or mice with myeloid C/EBPβ deficiency. Compared to wild-type cells, C/EBPβ-deficient macrophages underwent de-repression of CD38 expression in basal conditions. In contrast, CD38 induction by inflammatory cytokines, bacterial lipopolysaccharide or LXR agonists was drastically impaired in mice lacking C/EBPβ. Altogether, these results suggest crosstalk between LXRs and C/EBPβ to tightly regulate CD38 expression in the inflammatory context.
Grants: SAF2014-57856 from the Spanish Ministry of Economy and Competitivity and 080930 and 97/C/2016 from Fundació La Marató de TV3. The presenting author obtained an APIF fellowship from the University of Barcelona.

P.D4.10.09

CD4+ T cell mediated HLA class II cross-restriction in HIV controllers

M. Galperin[1], C. Farenc[2], M. Mukhopadhyay[1], J. Rossjohn[2], L. Chakrabarti[1], ***S. Gras***[2]*;*
[1]Pasteur Institute, Paris, France, [2]Monash University, Clayton, Australia.

Rare individuals, termed HIV controllers, spontaneously control HIV infection by mounting efficient T cell responses against the virus. Protective $CD4^+$ T cell responses from HIV controllers involve high affinity public T cell receptors (TCRs) recognizing an immunodominant capsid epitope (Gag293) presented by a remarkably broad array of HLA class II molecules. Here we determine the structures of a prototypical public TCR bound to HLA-DR1, -DR11, and -DR15 molecules presenting the Gag293 epitope. TCR recognition was driven by contacts with the Gag293 epitope, a feature that underpinned the extensive HLA cross-restriction. These high affinity TCRs promoted mature immunological synapse formation and cytotoxic capacity in both $CD4^+$ and $CD8^+$ T cells. The public TCRs suppressed HIV replication in multiple genetic backgrounds *ex vivo*, emphasizing the functional advantage conferred by broad HLA class II cross-restriction.

P.D4.10.10

Induction of neutrophil extracellular traps by *Mycoplasma bovis* and degradation of them by MnuA the major membrane exonuclease

F. Haile[1], *C. Hartley*[1], *F. Sansom*[1], *J. Coombe*[2], *P. Mansell*[2], *D. Beggs*[2], *G. Browning*[1];
[1]*Asia Pacific Centre for Animal Health, The University of Melbourne, Melbourne, Australia,* [2]*Department of Veterinary Clinical Sciences, Melbourne Veterinary School, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Melbourne, Australia.*

Mycoplasma bovis is recognised as an important pathogen of cattle and uses a range of cell surface proteins to evade the host immune system. We investigated the capacity of *M. bovis* to induce Neutrophil Extracellular Trap (NET) formation and the effect of the major membrane nuclease MnuA, which *in vitro* is responsible for the majority of the nuclease activity of *M. bovis,* on the process of NET formation. The wildtype *M. bovis* PG45, the nuclease deficient mutant MBOVPG45_0215 (*MnuA*), the putative nuclease Δ310 deficient mutant, and the *mnuA* complemented strain (*mnuA*-pIRR45) were grown in modified Frey's broth and their nuclease activities were compared using nuclease zymograms. Fluorescence microscopy was employed to visualise the presence of NETs in neutrophils isolated from healthy cows while a Sytox-Green based assay was used to quantify the formation of NETs. A luminol-based ROS assay was used to determine the role of reactive oxygen species (ROS) in the process of NET formation. NETs were detected following exposure of neutrophils to the *mnuA* mutant but not after exposure to either the wild-type or the *mnuA*-pIRR45 complemented mutant, and NETs were degraded in the presence of even low concentrations of wild type *M. bovis*. Our study demonstrates that *M. bovis* can induce NET formation in bovine neutrophils, albeit in the absence of induction of ROS, but that the major membrane nuclease MnuA is able to rapidly degrade them, and thus is likely to play a significant role in virulence.

P.D4.10.11

Unraveling the regulation of T cell - pathogen equilibration during chronic infection

J. Handschuh[1], *M. Alabdullah*[1], *P. Formaglio*[2], *L. Philipsen*[1], *J. Mohr*[1], *A. J. Müller*[1,3];
[1]*Institute for Molecular and Clinical Immunology, Magdeburg, Germany,* [2]*Institut Pasteur, Paris, France,* [3]*Helmholtz Centre for Infection Research, Braunschweig, Germany.*

Chronic infections require the escape of the pathogen from sterilizing immune responses, while the immune system may downregulate its effector functions to prevent damage at the cost of pathogen persistence. However, little is known about the mechanisms by which the immune response equilibrates with the pathogen in order to stabilize the infectious burden at a low level, which is key to permit chronic and often even asymptomatic infection. Particularly, it is unknown how the interactions of effector and regulatory T cells (Teff and Treg) among each other, as well with the pathogen, impact the establishment of a persisting pathogen reservoir.
We are using the intracellular parasite *Leishmania major* as a model for infections efficiently contained by T cells, yet persisting at the site of infection after resolution of pathology. The persisting parasite reservoir is important for maintenance of immunity against reinfection and is dependent on the presence of Treg.
We have set up in vivo biosensors in order to unravel the interplay of the T cell compartment and pathogen physiology at the site of infection by intravital 2-photon microscopy. This approach allows for probing not only pathogen viability, but also the activation of effector T cells during their dynamic interaction with *Leishmania*-infected phagocytes. By mapping immune cell activation and pathogen clearance in the context of the cellular microenvironment at the site of infection, we now aim at elucidating how the equilibrium between pathogen containment and immune activation is realized during the persistence of *Leishmania major*.

P.D4.10.12

Investigating the capacity of Kupffer cells to acquire an innate memory function through sensing of apoptotic cells

I. Liebold[1,2], *L. Bosurgi*[1,2];
[1]*University Hospital Eppendorf, Hamburg, Germany,* [2]*Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.*

Schistosoma are trematodes that are responsible for Schistosomiasis. Around 240 million people are infected with the parasite worldwide. Upon host infection, the release of parasite eggs leads to granuloma formation in the liver and induces a type 2 immune response. Macrophages, which react to type 2 cytokines, such as IL-4 and IL-13, are responsible and necessary for the switch from a type 1 to a type 2 immune response. Although this switch is essential for the initial survival of the host, at later stages of the disease, macrophages are also associated to the induction of liver fibrosis. Interestingly, Kupffer cells (KCs), resident macrophages in the liver, are not only highly phagocytic cells but they also have a long-lived capacity. Here we hypothesize that, similar to long lived cells of the adaptive immune system, KCs can acquire an innate memory capacity, driven by the phagocytosis of apoptotic cells. We have recently described that sensing of apoptotic cells is essential for the response to type 2 cytokines and acquisition of a tissue remodeling function in macrophages. Here we show that in the liver, Kupffer cells express phagocytic receptors such as AXL and MERTK, and can increase their tissue remodeling function upon sensing of apoptotic cells both in *in vitro* and *in vivo* experimental settings. Understanding new mechanisms for regulating, KCs activation and induction of tissue remodeling may reveal novel therapeutic targets and provide crucial insights for long lasting protection against hepatic infections, while avoiding liver fibrosis.
Founded by SFB841, Hamburg

P.D4.10.13

Ovine C-type lectin receptor (CLR)-Fc fusion protein library - a novel tool in veterinary immunology to screen for virus/CLR interactions

D. Lindenwald[1], *K. Jung*[2], *S. Becker*[3], *S. Rautenschlein*[4], *M. Buettner*[5], *G. Alber*[5], *B. Lepenies*[1];
[1]*Immunology Unit & Research Center for Emerging Infections and Zoonoses (RIZ), University for Veterinary Medicine Hannover, Foundation, Hannover, Germany,* [2]*Institute for Animal Breeding and Genetics & Research Center for Emerging Infections and Zoonoses (RIZ), University of Veterinary Medicine Hannover, Foundation, Hannover, Germany,* [3]*Institute for Parasitology & Research Center for Emerging Infections and Zoonoses (RIZ), University for Veterinary Medicine Hannover, Foundation, Hannover, Germany,* [4]*Clinic for Poultry, University for Veterinary Medicine Hannover, Foundation, Hannover, Germany,* [5]*Institute of Immunology/Molecular Pathogenesis, Center for Biotechnology and Biomedicine, College of Veterinary Medicine, University of Leipzig, Leipzig, Germany.*

Innate immunity is the first line of defense against parasitic, bacterial, fungal and viral pathogens and depends on the recognition of evolutionarily conserved pathogen patterns by innate immune receptors. Among other pattern recognition receptors, C-type lectin receptors (CLRs) recognize pathogen-derived patterns and thus are important cross-linkers of innate and adaptive immunity. Knowledge on how CLRs contribute to defense against infections is, however, incomplete. While to date most studies have focused on the role of CLRs in innate immunity in mice and men, little is known on the function of CLRs in larger animals. To this end, a library of chimeric CLR-Fc fusion proteins composed of the extracellular domain of ovine CLRs and human IgG Fc-fragment has been generated, thus extending the currently available murine and human CLR-Fc libraries by a veterinary relevant species.
The utility of the library was verified by ELISA-based binding studies. First, its functionality was confirmed using known ligands of the murine CLR paralogs, such as the Saccharomyces cerevisiae derived polysaccharide mannan as a ligand of ovine Dectin-1. Subsequent pathogen screenings revealed a selective affinity of some ovine CLR-Fc fusion proteins such as ovine Dendritic cell immunoreceptor (DCIR) to the contagious pustular dermatitis (ORF) virus.
In conclusion, the here generated ovine CLR-Fc fusion protein library represents a useful tool to screen for novel CLR/pathogen interactions in the field of veterinary immunology.

P.D4.10.15

The clinical efficacy of the combine interferon- and immunomodulatory therapy in patients with the atypical chronic active Epstein-Barr herpes-viral infection

I. V. Nesterova[1], *E. O. Khalturina*[2], *V. V. Malinovskaya*[3];
[1]*The Peoples' Friendship University of Russia, Moscow, Russian Federation,* [2]*I.M. Sechenov First Moscow State Medical University, Moscow, Russian Federation,* [3]*Federal Research Center of Epidemiology and Microbiology. N.F. Gamalei, Moscow, Russian Federation.*

Postviral chronic fatigue syndrome (pCFS) is often associated with atypical chronic active herpesviral Epstein-Barr infection (ACA-EBI). The methods of treatment of ACA-EBI are imperfect. We observed 148 adult patients suffering from ACA-EBI. The diagnostic complex included: the complaints, anamnestic, clinical features, the laboratory and immunological (the main cell'antiviral mechanisms, the interferon(IFN) system) investigations that was based on the our algorithm. Herpes viruses were tested by PCR and sero-diagnostic methods. All studied patients with ACA-EBI had mono- EBI (5%) and mixed EBI with another herpesviruses (95%). Symptoms of pCFS- in 97,97%, throat transient pain - in 100%, subfebrile temperature - in 67%, lymphadenopathy - in 90% of cases were identified. Secondary combine immunodeficiencies were detected: defect of induced production of INFα and INFγ - in 100% of cases; combine defects of immune system (number, functional deficiency of NK cells, T killers, EKT, neutrophils) - in 92.4% of patients. The program of combine IFN- and immunomodulatory therapy was created: suppositories of the recombinant INFα2 in combination with antioxidants, using gradually decreased doses - prolonged course (4.5 months); immunomodulatory therapy: glucosaminylmuramyl- dipeptide (GMDP), inosine pranobex and the synthetic antiherpesvirus drugs.
The course of combine IFN- and immunomodulatory therapy was lasted 4.5 month. The received clinical and immunological data clarified the immunopathogenetical mechanisms of ACA-EBI, that begun the basis for the development and application of targeted IFN-, immunomodulatory and antiviral therapy. Positive clinical and immunological dynamics was observed in 100% of cases, including the regression of CFS in 99% of patients.

P.D4.10.16

Analysis of the imbalance between regulatory B and T cells and circulating T follicular helper cells in HIV-infected patients

*J. Lopez-Abente[1], C. Gutierrez[2], V. Perez-Fernandez[1], A. Prieto-Sanchez[1], R. Correa-Rocha[1], S. Moreno-Guillen[3], M. Muñoz-Fernandez[1], **M. Pion**[1];*
[1]Instituto de Investigacion Sanitaria Gregorio Marañon, Madrid, Spain, [2]Hospital Ramón y Cajal, Madrid, Spain, [3]Hospital General Universitario Gregorio Marañón, Madrid, Spain.

Background: HIV infection *in vitro* produces alterations in regulatory T (Treg) and B (Breg) subsets. These subsets play a crucial role in the maintenance of immune homeostasis, and it has been recently described that T follicular helper cells (Tfh) are pivotal for the development of Breg, and could also modulate the Treg maintenance.
Methods: We have analyzed the phenotypes of four different Breg subsets, Treg and circulating Tfh (cTfh) compartments along with the frequencies of IL-10, IL-17, IL-4 and INFγ-secreting cells in naïve-treated HIV$^+$ patients, in treated-HIV$^+$ patients and in healthy individuals. Finally, we analyzed the suppressive capacity of Breg from HIV-infected patients or healthy individuals.
Results: Absolute counts of Treg and Breg were decreased and frequency of cTfh was increased in naïve-treated HIV$^+$ patients in comparison to treated-HIV$^+$ patients or healthy individuals. Positive correlation between cTfh and Treg observed in healthy individuals were lost in naïve-treatment HIV$^+$ patients, but surprisingly correlations between Breg subsets and Treg were established in naïve-treatment HIV$^+$ patients in comparison to healthy individuals. **Conclusions:** We demonstrated that the balance between cTfh, Treg and some Breg subsets are deregulated in HIV-infected patients and that these cellular compartments might participate in the immune system hyperactivation and exhaustion.
This work was supported by the Ministry of Economy and Competitiveness ISCIII-FIS grants PI12/01763, PI12/00934 and PI15/00923, co-financed by ERDF funds from the European Commission, "A way of making Europe". A.P-S and V. P-F were supported by the Youth Employment Program co-financed by the Madrid community and FEDER Founds.

P.D4.10.17

An unusual presentation of hip pain in a patient with known hyper IgE syndrome and multiple calcified pelvic apophyses

***A. Saad**, S. Shahban, T. ElGamal;*
Heart of England NHS trust, Birmingham, United Kingdom.

Introduction Hyper-IgE syndrome (HIES) is a relatively rare condition which, from childhood, renders patients susceptible to infection. Typically patients with HIES can develop various orthopaedic manifestations of this disease, namely, scoliosis, pathological fractures, osteoporosis and potentially septic arthritidies. Case report We present the case of WJ, a 44-year old patient with known HIES and a 7 week history of left hip pain. We discuss the clinical presentation, and the curveballs which came our way when investigating this patient and how we overcame them. We also demonstrate a very interesting pelvic radiograph from this patient which shows multiple sites of calcified apophyses. Something which is firstly unexpected in such patients and secondly something not previously reported in the literature. Conclusion Several issues and conundrums can present themselves when dealing with patients known to have HIES. We demonstrate how we managed such a patient and maintained a high level of suspicion in such patient.

P.D4.10.18

RNA derived from *Plasmodium falciparum* and *Litomosoides sigmodontis* is potent to induce a pro-inflammatory immune response in human cells

***J. F. Scheunemann**[1], A. L. Neumann[1], S. J. Frohberger[1], A. Ehrens[1], C. Coch[2], M. P. Huebner[1], A. Hoerauf[1,3], B. Schumak[1];*
[1]Institute of Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany, [2]Institute of Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, Bonn, Germany, [3]German Center for Infection Research (DZIF), partner site Bonn-Cologne, Germany.

Introduction: The elimination of infectious diseases like malaria and filariasis is an important aim of the United Nations. Despite tremendous research progress regarding the underlying mechanisms of these parasitic diseases, it still remains elusive how initial parasite recognition works. In contrast to surface structures and parasite products, the role of their nucleic acids is weakly characterized. Here we investigate the potential of parasitic nucleic acids and corresponding host receptors as targets for successful disease modification.
Materials and Methods: We investigated the potential of *P. falciparum* and *L. sigmodontis*-derived RNA to evoke an immune response, compared to human and *E. coli*-RNA, by *in vitro* cultures of human peripheral blood mononuclear cell (PBMC) and reporter cell lines. We quantified the secreted cytokines and analyzed cell activation by flow cytometry.
Results: ELISA revealed that cytosolic delivery of *L. sigmodontis*-RNA induced a strong induction of IFN-a in PBMCs, whereas *P. falciparum*-RNA was more stimulatory if delivered to the endosome, resulting in a cytokine pattern associated with NFκB activation. We further showed that monocytic cell populations were strongly activated after the challenge with parasitic RNA that was RIG-I-dependent.
Conclusion: Parasitic RNA derived from *P. falciparum* and *L. sigmodontis* harbors a pro-inflammatory potential, manifesting in the activation of monocytes, type I IFN secretion and presumably NFκB activation. The evoked immune response differs between endosomal and cytosolic recognition. The cytosolic RNA receptor RIG-I was crucial for recognition of parasitic RNA and subsequent cell activation.
Funding: Jürgen-Manchot-Stiftung (PhD scholarship JFS), BONFOR (JFS, BS), EXC1023 (JFS, CC, AH, BS)

P.D4.10.19

Reverse immunology as a tool to identify broadly recognized pneumococcal proteins targeted by human T-cells

***M. D. B. van de Garde**, E. van Westen, M. C. Poelen, N. Y. Rots, C. A. van Els;*
Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIV, Bilthoven, Netherlands.

Introduction: T-cell mechanisms, which are implied in protection against pneumococcal colonization, should be unraveled to understand the mode of action of future universal protein-based pneumococcal vaccines. Here we apply reverse immunology to predict and verify broadly recognized human T-cell epitopes for a semi-large array of pneumococcal proteins. Methods: Hundred pneumococcal proteins of diverging subcellular localization were selected for *in silico* prediction of T-cell immunogenicity based on HLA-DR binding and absence of cross-reactivity against human proteins (Epivax). For 20 potentially T-cell immunogenic proteins, peptides predicted to bind >4 of 8 common HLA-DRB1 alleles were synthesized, pooled per protein and tested in T-cell proliferation and cytokine assays using PBMCs from a panel of healthy adults and (ex-) pneumococcal pneumonia cases. Results: Peptide pools of 19/20 proteins evoked T-cell responses in healthy adults. Most frequent responses (in ≥ 25% of 20 donors tested) were found for SP_0117 (PspA), SP_0468 (putative sortase), SP_0546 (BlpZ), SP_1650 (PsaA), SP_1923 (pneumolysin), SP_2216 (PcsB), and SPR_0907 (PhtD). Healthy adults and cases had diverging patterns of protein immunodominance and cytokine profiles (IFNg, TNFa, IL-13 and IL17A) against single peptides. Conclusions: We demonstrated proof of principle for a reverse immunology approach to screen human pneumococcus specific T-cell responses at a semi-large proteome scale. Single peptides can evoke measurable proliferative and cytokine responses, including IL17, thought to play a role in the protection against *S. pneumoniae*. Currently, in depth T-cell analyses are ongoing in pneumococcal carriers and (ex-) cases of various age groups.

P.D4.11 Exploiting host pathogen interaction - Part 11

P.D4.11.02

HIV-1 modulates TRAF proteins to promote pro-inflammatory condition and avert Interferon induction

***S. Trivedi**, A. C. Banerjea;*
National Institute of Immunology, New Delhi, India.

The most obvious route for infection available to HIV-1 in humans is via epidermis, dermis and lymph nodes and doing so it encounters a lot of resistance as every nucleated cell it comes across can potentially mount an innate immune response against it. Especially, the tissue dendritic cells and macrophages where the virus faces various pattern recognition receptors (PRRs) before it can establish a productive infection. An important family of signaling adaptor proteins involved in different PRR pathways is TRAF (TNF-α Receptor Associated Factor) Family of proteins. Almost all PRRs involved in detection of HIV-1 converge down to TRAF family of proteins which in turn leads to differential effecter functions. On one hand, TRAF6 activates Nf-κB and AP-1, which binds to the LTR region of HIV-1 and promotes its transcription, while on the other hand, TRAF3 activates IRF-3 and IRF-7 which promote synthesis of interferons and in turn provide anti-viral activity. Thus modulation of these two effecter arms at the right time can be very beneficial for the virus and delineating this phenomenon can provide better insights into the pathogenicity of the virus. In our work we are able to show that HIV-1 modulates these key signaling adaptors - TRAF3 and TRAF6 to enhance its replication capacity in the cell. HIV-1 Vpr and Vpu proteins are involved in this modulation which down-regulate TRAF3 and up-regulate TRAF6 to a similar extent, thereby creating a pro-inflamatory environment which enables the virus to have a much more productive replication.

P.D4.11.03

Suppressor of cytokine signaling-3 and control of progression towards liver fibrosis and hepatocellular carcinoma in chronic HCV-infected patients

F. JADID[1,2], *H. Chihab*[1], *H. Salih Alj*[2], *R. Elfihry*[1], *I. zaidane*[1], *S. Tazi*[1], *W. Badre*[3], *A. Marchio*[4], *M. Tahiri*[3], *R. Saile*[2], *P. Pineau*[4], *S. Ezzikouri*[1], *S. Benjelloun*[1];
[1]*Institut Pasteur du Maroc, Casablanca, Morocco,* [2]*Université Hassan II de Casablanca, Casablanca, Morocco,* [3]*CHU Ibn Rochd de Casablanca, Casablanca, Morocco,* [4]*Institut Pasteur de Paris, Paris, France.*

Chronic Hepatitis C is one of the most important risk factors of liver cirrhosis and hepatocellular carcinoma. Before reaching these ultimate steps, insulin resistance triggered by hepatitis C virus infection is known to participate in the progression of liver disease. The present study aims to investigate the influence of two functional polymorphisms on *SOCS3* mRNA expression and on the outcomes of CHC progression in a North African context. In this case-control study, 601 Moroccan subjects composed of 200 healthy controls, 101 resolvers and 300 patients with persistent HCV infection including 95 mild chronic hepatitis, 131 Advanced Liver Diseases and 74 HCC were enrolled. They were genotyped for the 4874 A/G (rs4969170) and A+930-->G (rs4969168) *SOCS3* variants using TaqMan SNPs assays. SOCS3 mRNA expression was assessed using Real Time PCR technique. Logistic regression analysis showed that variation at rs4969168 was associated with spontaneous clearance of HCV (P<0.05). In addition, minor allele frequencies were significantly higher in AdLD patients when compared to the mCHC group both for rs4969168 (P=7.0 E-04) and rs4969170 (P=4.0 E-05). A significant association between haplotype and liver disease progression was also found. Moreover, SOCS3 mRNA was significantly more expressed in peripheral leukocytes from patients with HCC than in those from mCHC. Finally, rs4969170 was significantly associated with LDL-lipoprotein (P=0.04), total cholesterol (P=5.0 E-04), and higher fasting glucose levels (P=0.005) in patients with persistent HCV infection. Our results underline the importance of the functional SOCS3 polymorphisms in the modulation of CHC progression and suggest their contribution to HCC development by affecting its mRNA expression and perturbing key metabolic parameters.

P.D4.11.04

PIP_3 induces phagocytosis of non-motile *Pseudomonas aeruginosa*

S. Demirdjian*, D. Hopkins, H. Sanchez, B. Berwin;*
Dartmouth College, New Hampshire, United States.

Pathogenic bacteria that establish chronic infections in immunocompromised patients frequently undergo adaptation or selection for traits that are advantageous for their growth and survival. Clinical isolates of *Pseudomonas aeruginosa*, a gram-negative, opportunistic bacterial pathogen, exhibit a temporal transition from a motile to a non-motile phenotype through loss of flagellar motility during the course of chronic infection. This progressive loss of motility is associated with increased resistance to both antibiotic and immune clearance. We have previously shown that loss of bacterial motility enables *P. aeruginosa* to evade phagocytic clearance both *in vitro* and *in vivo* and fails to activate the phosphatidylinositol 3-kinase (PI3K)/Akt-dependent phagocytic pathway. Therefore, we tested the hypothesis that clearance of phagocytosis-resistant bacteria could be induced by exogenously pre-treating innate immune cells with the Akt activating molecule phosphatidylinositol-(3,4,5)-trisphosphate (PIP_3). Here we demonstrate that PIP_3 induces the uptake of non-motile *P. aeruginosa* by primary human neutrophils >25-fold, and this effect is phenocopied with the use of murine phagocytes. However, surprisingly, mechanistic studies revealed that the induction of phagocytosis by PIP_3 occurs because polyphosphoinositides promote bacterial binding by the phagocytes rather than bypassing the requirement for PI3K. Moreover, this induction was selective, since the uptake of other non-motile gram-negative, but not gram-positive bacteria, can also be induced by PIP_3. Since there is currently no treatment that effectively eradicates chronic *P. aeruginosa* infections, these findings provide novel insights into a potential methodology by which to induce clearance of non-motile pathogenic bacteria and into the endogenous determinants of phagocytic recognition of *P. aeruginosa*.
NIH (P30 RR032136-01, R21 AI121820), Cystic Fibrosis Foundation (STANTO19R0, STANTO11R0).

P.D4.11.05

Estrogen stimulates phagocytosis by macrophages in both *in vitro* and *ex vivo* models of age-related impaired healing via the estrogen-receptor alpha

M. El Mohtadi*, K. Whitehead, A. Fadel, J. Ashworth;*
School of Healthcare Science, Manchester, United Kingdom.

Annual expenditure for the treatment of chronic wounds in the elderly exceeds $9 billion. Chronic wounds are frequently colonised by opportunistic pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and declining levels of estrogen with increasing age delays healing. This study investigated the effect of hormonal aging (estrogen deprivation) on the clearance of methicillin-resistant *S. aureus* (MRSA) and *P. aeruginosa* by macrophages derived from U937 and human primary $CD14^+$ monocytes. Concentrations of 17β-estradiol were used to model estrogen levels found in the elderly (estrogen deprivation: absolute absence and $1x10^{-9}$M), young adults ($1x10^{-8}$M) and following exogenous supplementation ($1x10^{-7}$M). The estrogen receptor (ER) isoform(s) involved in bacterial clearance were determined using selective ER modulators.
Estrogen at concentrations typical of youth or supraphysiological levels significantly ($P<0.05$; n=24) increased the phagocytosis of MRSA and *P. aeruginosa* in a concentration-dependent manner compared to estrogen deprivation. Confocal and scanning electron microscopy confirmed estrogen increases co-localisation of fluorescent GFP-*S. aureus* or mCherry-*P. aeruginosa* with macrophages and promotes bacterial internalisation. ER-alpha (ERα) activation mirrored the stimulatory effect of estrogen on phagocytosis whilst ERα antagonism completely blocked the effect of estrogen. In contrast, activation or antagonism of ER-beta (ERβ) had no effect on phagocytosis, confirming estrogen mediates bacterial clearance via ERα alone.
These findings suggest estrogen promotes the resolution of wound bacteria during youth but this protection is lost as estrogen levels decline with increasing age. Novel dressings that provide estrogen supplementation or selective activation of ERα may be an effective treatment option for colonised wounds in the elderly.

P.D4.11.06

Chlamydial co-infection boosts the CTL-stimulatory capacity of HIV-1-exposed DCs in a time-dependent manner

M. Schönfeld[1], *U. Knackmuss*[1], *P. Chandorkar*[1], *P. Hörtnagl*[2], *T. J. Hope*[3], *A. Moris*[4], *R. Bellmann-Weiler*[5], *C. Lass-Flörl*[1], *D. Wilflingseder*[1], ***W. Posch***[1];
[1]*Division of Hygiene and Medical Microbiology, Medical University Innsbruck, Innsbruck, Austria,* [2]*Central Institute for Blood Transfusion & Immunological Department, Innsbruck, Austria,* [3]*Department of Cell & Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago, United States,* [4]*Centre d'Immunologie et des Maladies Infectieuses-Paris, Pierre and Marie Curie University UMRS C7, INSERM U1135, CNRS ERL 8255, Paris, France,* [5]*University Hospital for Internal Medicine II, Medical University of Innsbruck, Innsbruck, Austria.*

Pathogenic bacteria and their microbial products activate dendritic cells (DCs) at mucosal surfaces during sexually transmitted infections (STIs) and might also modulate their functions during co-infections with HIV-1. We recently illustrated that complement (C) coating of HIV-1 (HIV-C) as found during the acute phase of infection by-passed SAMHD1-mediated restriction in DCs and thereby mediated an increased DC activation and antiviral capacity. To determine whether the superior antiviral effects of HIV-C-exposed DCs also apply during bacterial co-infection, we developed a super-infection model in which DCs were infected with *Chlamydia spp.* simultaneously (HIV+Chlam-DCs) or followed by HIV-1 infection (Chlam-DCs). Simultaneous infection of DCs with HIV-1 and *Chlamydia* significantly boosted the CTL-stimulatory capacity compared to HIV-C-loaded iDCs. This protective effect was lost upon pre-infection with *Chlamydia* 3h or 24h prior addition of opsonized HIV-1. The reduction in the CTL-stimulatory capacity was not due to lower HIV-1 binding, internalization or infection of Chlam-DCs compared to iDCs or HIV+Chlam-DCs, but due to altered fusion and internalization mechanisms within DCs. CTL-stimulatory capacity of HIV-C in Chlam-DCs correlated with significantly reduced viral fusion than iDCs and HIV+Chlam-DCs and illustrated considerably increased numbers of HIV-C-containing vacuoles compared to iDCs. These data indicate that *Chlamydia* super-infection of DCs mediates a transient boost of their HIV-specific CTL-stimulatory and antiviral capacity, which is reversed in a time-dependent manner.

P.D4.11.07

Hyperinduction of Interferon Lambda and proinflammatory cytokines upon infection of neural cells with Zika virus

A. S. M. SELIM*, S. M. Lee;*
The University of Hong Kong, Hong Kong, Hong Kong.

In Feb, 2016, The WHO has declared Zika virus as a "Public Health emergency of international concern" after the outbreak in Brazil where numerous cases of microcephaly in newborns of Zika infected mothers as well as acute myelitis in adults. Zika is an enveloped, non-segmented, positive sense, single stranded RNA arbovirus. Here we investigated the viral replication kinetics and the host immune response in human neural cells after Zika virus infection. Human differentiated astrocytes (d T98G), neuronal (d SH-SY5Y) and microglial (Immortalized Human Microglia-SV40) cells were infected with Zika virus at MOI of 4. TCID50 was used to measure the viral replication kinetics. We found that Zika replicated efficiently in the neuronal and microglial cells but to a lesser extent in astrocytes. RT-qPCR was used to measure the host immune gene response after infection. We found that Zika induced type I interferon represented in IFN-beta induction in microglial and astrocytic cells. Interestingly, we found that Zika induced the expression of type III interferons, IL29 in astrocytic and microglial cells and IL28a/b in neuronal and microglial cells as well as IFN λ receptor (IL10RB). Proinflammatory cytokines and chemokines hyper inductions were sparking with RANTES, IP10, IL8, IL6 and COX2. Hyperinductions of pattern recognition receptors involved in viral recognition were identified with TLR3 and RIG-I. Our results demonstrated that three of the main neural cell types are susceptible to Zika virus infection. The hyper- induction of IL-29 in astrocytic and microglial cells opens new insights for Zika host antiviral response.

P.D4.11.08

Blood transcriptomic profiles to differentiate enteroviral meningitis from bacterial meningitis

E. Bartholomeus[1,2], *N. De Neuter*[3,4,2], *A. Lemay*[5], *D. Tuerlinckx*[6], *D. Weynants*[7], *K. Van Lede*[8], *G. Van Berlaer*[9], *T. Boiy*[10], *A. Vander Auwera*[11], *M. Raes*[12], *D. Van Der Linden*[13], *S. Van Steijn*[14], *T. Jonckheer*[15], *H. Verhelst*[16], *A. Suls*[1,2], *P. Vandamme*[17], *K. Laukens*[18,4,2], *G. Mortier*[1,2], *P. Meysman*[18,4,2], *B. Ogunjimi*[10,19,2];
[1]*Center of Medical Genetics, Edegem, Belgium,* [2]*Antwerp Unit for Data Analysis and Computation in Immunology and Sequencing, University of Antwerp, Antwerpen, Belgium,* [3]*Adrem Data Lab, University of Antwerp, Wilrijk, Belgium,* [4]*Biomedical Informatics Research Network Antwerp (biomina), University of Antwerp, Antwerpen, Belgium,* [5]*AZ Turnhout, Turnhout, Belgium,* [6]*CHU-ULC Dinant/Godinne, Dinant, Belgium,* [7]*CHU-ULC Namur Ste Elisabeth, Namur, Belgium,* [8]*AZ Nikolaas, Sint-Niklaas, Belgium,* [9]*Universitair Ziekenhuis Brussel, Brussels, Belgium,* [10]*Antwerp University Hospital, Edegem, Belgium,* [11]*GZA Sint-Augustinus, Wilrijk, Belgium,* [12]*Jessa Hospital, Hasselt, Belgium,* [13]*CHU-ULC Saint-Luc, Brussels, Belgium,* [14]*ZNA Paola, Antwerpen, Belgium,* [15]*GZA Sint-Vincentius, Antwerpen, Belgium,* [16]*Ghent University Hospital, Ghent, Belgium,* [17]*Centre for the Evaluation of Vaccination (CEV), Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerpen, Belgium,* [18]*Adrem Data Lab, University of Antwerp, Antwerpen, Belgium,* [19]*Centre for Health Economics Research & Modeling Infectious Diseases (CHERMID), Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerpen, Belgium.*

Introduction: Meningitis can be caused by several viruses and bacteria. Identifying the specific pathogen as fast as possible is crucial to initiate the most optimal therapy, as acute bacterial meningitis can be fatal. Bacterial meningitis requires antibiotics, as opposed to enteroviral meningitis, which only requires supportive therapy. Clinical presentation is mostly not sufficient to differentiate between viral and bacterial meningitis, thereby necessitating cerebrospinal fluid (CSF) analysis by PCR and/or time-consuming bacterial cultures. However, collecting CSF in children is not without risk.
Methods: In 11 Belgian hospitals, we obtained acute blood samples from children with signs of meningitis (3 months – 16 years). When meningitis was confirmed on CSF, the patient was asked to give a convalescence sample after recovery. 3'mRNA sequencing was performed to determine differential expressed genes to create a host transcriptomic profile. Gene ontology analysis was used for further interpretations.
Results: Enteroviral meningitis cases displayed the largest upregulated fold change enrichment in interferon production (especially interferon-alpha), response and signaling pathways when comparing acute with convalescence samples. Furthermore, we noted an upregulation of expression of different other cytokines, activation and degranulation of leukocytes, including neutrophil mediated immunity. We compared enteroviral against bacterial meningitis data and built a random forest classifier with feature selection with an AUC of 0,957.
Conclusion: Enteroviral meningitis has a clear innate immunity signature with type 1 interferons as key players, as expected in a viral infection. Our classifier, based on the host transcriptomic profiles of different meningitis cases, is able to select for enteroviral meningitis.

P.D4.11.09

Dissecting the molecular mechanisms underpinning T cell help for dendritic cell

E. Gressier[1,2], *P. G. Whitney*[1], *W. Kastenmuller*[2], *S. Bedoui*[1];
[1]*The Peter Doherty Institute for Infection and Immunity, Melbourne, Australia,* [2]*Institute of Experimental Immunology, Bonn, Germany.*

$CD8^+$T cell priming relies on the ability of dendritic cells (DC) to present antigen as well as recognizing, processing and communicating contextual cues associated with antigen acquisition to $CD8^+$T cells. DC often require CD40-CD40L-mediated interactions with helper $CD4^+$T cells to generate an efficient $CD8^+$T cell response. Precisely how such 'T cell help' is delivered *in vivo* and how it optimizes the priming capacity of DC remains unclear. Previous work conducted by the team showed that $CD4^+$T cell help amplifies the capacity of DCs to generate efficient priming of $CD8^+$T cell in response to suboptimal innate stimuli. Focusing on the CD40-driven amplification of innate pathways induced in IFN-α/β-stimulated DC, this study was designed to explore the molecular mechanisms underpinning such help phenotype. We have observed distinct patterns and dynamics of a fast amplification of various cytokines such as IL-15, IL-6 and IL-12p40, amplification induced by CD40 treatment following IFN-α stimulation. Moreover, transcriptomics, proteomics and protein phosphorylation analysis suggest that this crosstalk between interferon and T cell help signal was integrated through the distinct stimulation of particular aspects of the NF-κB pathways. These findings argue for a complex synergism between CD40 signaling and innate stimuli that enable DCs to flexibly adjust cytokine secretion to the strength of inflammatory responses that accompany the acquisition of antigen. By dissecting how T cell help amplifies innate signals required for $CD8^+$T cell priming, this work will assist in the development of more targeted T-cell-based therapeutic strategies.

P.D4.11.10

Adrenal hormones mediate disease tolerance in malaria

L. Vandermosten[1], *T. Pham*[1], *S. Knoops*[1], *C. De Geest*[1], *N. Lays*[1], *K. Van der Molen*[1], *C. J. Kenyon*[2], *M. Verma*[2], *K. E. Chapman*[2], *F. Schuit*[3], *K. De Bosscher*[4], *G. Opdenakker*[1], ***P. E. Van den Steen***[1];
[1]*Rega Institute for Medical Research, KU Leuven - University of Leuven, Leuven, Belgium,* [2]*The Queen's Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom,* [3]*KU Leuven - University of Leuven, Leuven, Belgium,* [4]*VIB Center for Medical Biotechnology, Ghent University, Ghent, Belgium.*

Malaria, a global parasitic disease with severe complications, reduces host fitness and survival by pathogen-mediated damage and/or exaggerated host inflammation. Disease tolerance mechanisms counter these negative effects without interfering with pathogen load and thereby improve host survival. Adrenal hormones, including glucocorticoids (GCs) and adrenalin, have several homeostatic functions. GC levels are increased in patients with malaria, but their precise role remains unknown. In four different mouse models of malaria, we demonstrated that adrenal hormones protect mice against early death during infection, independent of parasitemia and parasite-mouse strain combinations. Adrenal hormones thus confer disease tolerance in malaria. Surprisingly, adrenalectomy differentially affected malaria-induced inflammation by increasing circulating cytokine levels and inflammatory activity in the brain, but not in the liver or lungs. Furthermore, without effects on the hepatic gluconeogenic enzyme transcription and the free fatty acid levels in plasma, adrenalectomy caused lethal hypoglycemia upon infection, independently from TNF-α and insulin. Glucose administration did not prevent or reverse lethal hypoglycemia. In contrast, treatment with a synthetic GC (dexamethasone) prevented the hypoglycemia, lowered cerebral cytokine expression and significantly increased the survival rates. Overall, we conclude that adrenal hormones do not protect against lung and liver inflammation in malaria, but instead protect against systemic and brain inflammation and severe hypoglycemia.
Funding: Research Foundation-Flanders (F.W.O.-Vlaanderen) and the Research Fund (Geconcerteerde Onderzoeksacties GOA 2013/014 and C1 project C16/17/010) of KU Leuven. TP holds an aspirant PhD fellowship of the F.W.O.-Vlaanderen and PVdS is a Research Professor at the KU Leuven.

P.D4.11.11

TOB1 inhibits IRF3-directed antiviral responses by recruiting HDAC8 to specifically suppress IFN-β expression

Z. Yu, M. Jia, ***W. Zhao****;*
Department of Immunology,School of Basic Medical Science, Shandong University, China, Jinan, China.

Innate immunity is the first line of host to defense against viral invasion and need to be precisely controlled. Viral infection induced type I interferons (IFNs) production play fundamental roles in innate immunity against virus and maintain immune homeostasis. However, the epigenetic regulatory mechanisms of type I IFNs production is unclear. The transducer of ErbB-2.1 (TOB1) is a member of the anti-proliferative family of BTG/TOB (B cell migration factor/erbB2). TOB1 plays crucial regulatory roles in T cell activation and cancer, via interacting with SMAD4 or SMAD2. However, its potential roles in innate immunity is unknown. In the present study, we found that the crystal structures of SMAD and IRF3 are very similar, and TOB1 could interact with IRF3. TOB1 expression could be markedly induced during viral infection in macrophages. TOB1 deficiency enhances both RNA and DNA viral-induced IFN-β production, and inhibits viral replication in macrophages. Mechanistically, TOB1 associates with IRF3, recruits HDAC8, and promotes HDAC8 binding to IFN-β promoter. Thus, TOB1 attenuates the acetylation of histone in the IFN-β promoter region, and thus inhibiting IFN-β transcription. Therefore, we identified TOB1 as a negative regulator of IFN-β production and outlined a new feedback mechanism for the epigenetic control of antiviral immune responses.

P.D4.11.12

Schlafen 14 (SLFN14) is a novel antiviral factor involved in the control of viral replication

O. Shin*;*
Korea University School of Medicine, Seoul, Korea, Republic of.

Schlafen (SLFN) proteins have been suggested to play important functions in cell proliferation and immune cell development. In this study, we determined the antiviral activities of putative RNA-helicase domain-containing SLFN14. Murine SLFN14 expression was specifically induced by TLR3-mediated pathways and type I interferon (IFN) in RAW264.7 mouse macrophages. To examine the role of SLFN during viral infection, cells were infected with either wild-type PR8 or delNS1/PR8 virus. SLFN14 expression was specifically induced following influenza virus infection. Overexpression of SLFN14 in A549 cells reduced viral replication, whereas knockdown of SLFN14 in RAW264.7 cells enhanced viral titers. Furthermore, SLFN14 promoted the delay in viral NP translocation from cytoplasm to nucleus and enhanced RIG-I-mediated IFN-β signaling. In addition, SLFN14 overexpression promoted antiviral activity against varicella zoster virus (VZV), a DNA virus. In conclusion, our data suggest that SLFN14 is a novel antiviral factor for both DNA and RNA viruses.

P.D4.11.13

Th17 cells and the adaptive immunity to vulvovaginal candidiasis

R. Marzi[1], *C. Basso*[1], *C. De Gregorio*[1], *F. Kirchner*[2], *G. Gyuelveszi*[1,3], *S. Becattini*[1,4], *B. Becher*[5], *S. LeibundGut-Landmann*[2], *F. Sallusto*[1,6];
[1]*Institute for Research in Biomedicine,Università della Svizzera Italiana, Bellinzona, Switzerland,* [2]*Section of Immunology, Institute of Virology, University of Zurich, Zurich, Switzerland,* [3]*Roche Innovation Center Zurich, Zurich, Switzerland,* [4]*Memorial Sloan Kattering Cancer Center, New York, United States,* [5]*Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland,* [6]*Institute of Microbiology, ETH Zurich, Zurich, Switzerland.*

Vulvovaginal candidiasis (VVC) is the most common manifestation of *C. albicans* infection. Chronic mucocutaneous candidiasis (CMC) patients with mutations in genes associated with the IL-17 pathway display symptoms of VVC, but whether the disruption of the IL-17 pathway is a direct cause for VVC or rather an indirect effect due to the general increase in fungal colonization in these patients is unclear. Mice lacking IL-23, either IL-17R subunit or the adaptor Act1, are susceptible to oral and dermal candidiasis. These previous studies analyzed only the early phases of the host-pathogens interplay, which is generally life-long in humans. In our study, we set out kinetic experiments to assess the relative contribution of Th17 cells in persistent VVC. We found that WT mice and μMT mice survived even at later time points and completely recovering from the infection, while CD3ε$^{-/-}$ mice succumbed to the challenge. In order to define the mechanism of T cell-mediated protections, we adoptively transferred CD3ε$^{-/-}$ mice with *Candida*-specific TCR-transgenic T cells. *Candida*-specific CD4$^+$ *in vitro* polarized Th17 cells confer protection to VVC and using IL-17A$^{-/-}$ mice we highlighted that IL-17A, produced by T cells, is required for long-term containment of infection. On the contrary, Th2 cells have a detrimental effect on Th17 cells affecting their expansion, probably through IL-4 production since anti-IL-4 treatment restores Th17 ability to control infection. Our results suggest that CD4$^+$ T cells are required for long-term control of VVC and in particular only IL-17-producing Th17 cells confer protection against *C. albicans* infection.

P.D4.11.14

Androgens inhibit phagocytosis by macrophages via the androgen receptor

C. Parry, ***M. El Mohtadi****, K. Whitehead, J. Ashworth;*
School of Healthcare Science, Manchester, United Kingdom.

The cost to healthcare services for the treatment of chronic wounds in the elderly exceeds $9 billion per annum. Chronic wounds are frequently colonised by opportunistic nosocomial pathogens such as *Staphylococcus aureus*. Endogenous androgens are known to contribute to delayed healing whilst in contrast, estrogen accelerates healing. Healing in the elderly is impaired, particularly in elderly males due to the concomitant decline in estrogen levels yet largely unchanged levels of circulating androgens. This study investigated the effect of the two main endogenous androgens, testosterone (T) and dihydrotestosterone (DHT), on the clearance of methicillin-resistant *S. aureus* (MRSA) by macrophages derived from U937 monocytes. Concentrations of T and DHT were chosen to model adult physiological ranges of androgen levels (1x10^{-9}M, 1x10^{-8}M, 1x10^{-7}M) and supraphysiological levels following exogenous supplementation (1x10^{-6}M). The involvement of androgen receptor (AR) activation in bacterial clearance was confirmed using AR antagonists. Concentrations of T and DHT typical of adults or supraphysiological levels significantly ($P<0.05$; n=30) inhibited phagocytosis of MRSA in a concentration-dependent manner compared to untreated controls. Confocal and scanning electron microscopy confirmed androgens decrease the co-localisation of fluorescent GFP-*S. aureus* with macrophages and inhibit bacterial internalisation. AR antagonism reversed the effect of T and DHT, significantly ($P<0.05$) enhancing phagocytosis. These findings suggest androgens inhibit the resolution of wound bacteria. Novel dressings that induce local blockade of the AR may be an effective treatment option to promote the bacterial clearance of colonised wounds, particularly in elderly males.

P.D4.11.15

Effect of BCG and rBCGmIL-18 on central and effector memory T cells in C57BL/6 mice in the model of immunosuppression induced by cyclophosphamide

M. Kowalewicz-Kulbat*, K. Krawczyk, M. Wlodarczyk, M. Druszczynska, W. Rudnicka, M. Fol;*
Department of Immunology and Infectious Biology, Lodz, Poland.

Introduction Memory T cells can provide long-term immunity. Cyclophosphamide (CTX) is an immunosuppressive agent used in cancer therapy. Still little is known how CTX may act on the memory T-cell population and whether BCG bacilli can modify this process. **Aim** The aim of our study was to compare the response of central (T_{CM}) and effector memory T cells (T_{EM}) in immunized with *Mycobacterium bovis* BCG and recombinant BCG producing murine IL-18 (rBCGmIL-18) C57BL/6 mice (sensitive to mycobacterial infection), under immunosuppressive conditions. **Materials and methods** Mice were intradermally immunized with BCG or rBCGmIL-18 and after 6 and 12 weeks intraperitoneally treated with CTX (50μg/g b.w.) for 7 days. The splenocytes were isolated and stained with the monoclonal antibodies to determine the expansion of T_{CM} expressing CD62L$^+$CD44$^{+/-}$ CD127$^+$CD4$^+$ and T_{EM} with phenotype CD62L$^-$CD44$^+$CD127$^{+/-}$CD4$^+$. The percentage of the T_{CM} and T_{EM} was determined by flow cytometry. **Results** We observed a strong inhibition of CD4$^+$ T cell population in immunosuppressed mice immunized for 12 weeks with BCG and rBCGmIL-18 compared to mice immunized for 6 weeks. Interestingly, rBCGmIL-18 in immunosuppressed mice significantly increased the amount of CD4$^+$ CM compared to BCG in mice immunized for 12 weeks but not 6 weeks. This effect was not observed in CD4$^+$EM population in mice immunized with mycobacteria for 6 and 12 weeks. **Conclusion:** Our results indicate that under immunosuppressed conditions, rBCGmIL-18 displayed better ability than BCG to increase the percentage the CD4$^+$$T_{CM}$ without an effect on CD4$^+$$T_{EM}$ cells what may extend the knowledge in understanding the protective response of memory T cells in immunosuppressed conditions. Grant NCN 2013/11/B/NZ6/01304

P.D4.11.16

Immune phenotype related to coinfection in patients admitted to the emergency department for influenza virus infection

A. C. HERNANDEZ PADILLA[1], *R. Jeannet*[2], *T. Lafon*[3], *E. Lereclus*[4], *O. Barraud*[5], *S. Hantz*[6], *A. Fedou*[7], *M. Goudelin*[8], *B. Evrard*[8], *A. Desachy*[8], *P. Vignon*[9], *T. Daix*[10], *B. François*[10];
[1]*Inserm CIC 1435, Limoges, France,* [2]*CNRS UMR 7276/INSERM U 1262, Université de Limoges, Limoges, France,* [3]*Inserm CIC 1435/Service d'Accueil des Urgences, CHU Dupuytren, Limoges, France,* [4]*UMR CNRS 7276 / UMR INSERM 1092 Centre de Biologie et de Recherche en Santé (CBRS), Limoges, France,* [5]*Inserm UMR 1092 / Laboratoire de Bactériologie-Virologie-Hygiène, Université de Limoges/CHU Dupuytren, Limoges, France,* [6]*Inserm UMR 1092 / Laboratoire de Bactériologie-Virologie-Hygiène, Université de Limoges / CHU Dupuytren, Limoges, France,* [7]*Réanimation Polyvlente, CHU Dupuytren, Limoges, France,* [8]*Réanimation Polyvalente, CHU Dupuytren, Limoges, France,* [9]*Réanimation polyvalente / Inserm CIC 1435, CHU Dupuytren / Université de Limoges, Limoges, France,* [10]*Réanimation polyvalente / Inserm CIC 1435 / Inserm UMR 1092, CHU Dupuytren / Université de Limoges, Limoges, France.*

Introduction: Influenza virus infection (IVI) outbreaks are associated to high morbidity and complications. Coinfection is a risk factor for mortality in IVI. Immune dysregulation secondary to IVI is known to play a role in Acute Respiratory Distress Syndrome (ARDS) and mortality, but its role in coinfection has not been sufficiently explored. This study aimed at characterizing the differential immune phenotype of patients admitted to the emergency department (ED) for IVI according to associated coinfection. **Methods:** A prospective, observational study was performed in adult patients admitted to our ED with IVI confirmed by PCR from nasopharyngeal swabs between January and March 2018. Patients with history of malignancies or immunosuppression were excluded. Flow cytometry from peripheral blood was performed to determine T cell, monocyte and granulocyte subsets and activation status at admission. **Results:** 26 patients with Influenza A pdm2009H1 (65%) or Influenza B (35%) (54.5 years old, IQR 39.3-62; 10 men) were included. 50% required ICU admission and 31% had pulmonary coinfection during hospital stay. 4 patients (15%) died, all of them with documented coinfection. At admission, lymphocytes (1.08 vs. 2.57 G/L; p=0.004), HLA-DR+ monocytes (22.28% vs. 61.94%; p=0.006 and 0.0023 vs. 0.1982 G/L; p=0.007), and activated monocytes (0.045 vs. 0.1892 G/L; p=0.009) were decreased in later coinfected patients; while immature granulocytes (26.52% vs. 4.06%; p=0.049), and M-MDSC (65.54% vs. 31.99%; p=0.016) were increased. **Conclusions:** Innate and adaptive immune dysregulation related to IVI leads to decreased mature, effective lymphocyte, monocyte and granulocytes counts. It seems to be a mechanism facilitating coinfection, and could serve as early predictor of such outcome.

P.D4.11.17

Deregulation of monocyte related cytokine/chemokine profile in serum milieu during Puumala virus infection

L. Cvetko Krajinovic[1], *P. Svoboda*[1], *A. Topić*[1], *P. Čikeš*[1], *M. Bosnar*[2], *V. Eraković Haber*[2], *A. Markotić*[1];
[1]*University Hospital for Infectious Diseases, Zagreb, Croatia,* [2]*Fidelta Ltd, Zagreb, Croatia.*

Changes in circulating cytokine and chemokine levels have been associated with many human diseases, and thus understanding the relationships between these changes and disease is an important. Hemorrhagic fever with renal syndrome (HFRS) is a viral disease caused by hantaviruses, however, it is primarily considered as an immune-mediated disease. Several studies have shown deregulated pro- and anti-inflammatory cytokine/chemokine production during the acute phase of hantavirus infection in patients' sera. Monocytes/macrophages are important producers of different cytokines/chemokines significantly contributing to the status of the serum milieu.
They are also considered as one of the target cells for hantaviruses. The aim of our study was to analyze kinetics of the monocytes/macrophages related cytokines/chemokines in HFRS patients' serum during the course of disease and correlate the serum profile with the severity of the clinical outcome of Puumala virus (PUUV)-induced disease. Serum samples were collected two times during the disease course. The mean levels of 16 cytokines and chemokines were determined by multiplex immunoassay with magnetic beads. When compared to the controls, some pro-inflammatory cytokines were unaltered (IL-1β, IL-12(p70), IL-23) due to infection, whereas levels of CCL5 and TGF-β1 were significantly lower in infected cases. Significantly elevated levels of IL-15, IL-1RA, IL-18, MIF, CCL2 and CCL4 were detected in PUUV-infected individuals. We found no significant differences in the levels of cytokines/chemokines between the group of patients with moderate symptoms compared to individuals with mild symptoms but cytokines like CCL5 and TGF-β1 showed strong positive correlations with some HFRS-associated pathological laboratory findings.

P.D4.11.18

Dengue virus infects epidermal human Langerhans cells for transmission to dermal dendritic cells

***L. C. Helgers**, J. K. Sprokholt, T. B. Geijtenbeek;*
AMC, Amsterdam, Netherlands.

Dengue virus (DENV) is an enveloped positive ssRNA *flavivirus* that infects 390 million people on an annual basis. During recent years, studies have focused on DENV infection of dendritic cells (DC), macrophages and monocytes. However, DENV is introduced into the human epidermis via mosquitos bites. Therefore, a specialized subset of epidermal DCs called Langerhans cells (LCs) might be a target for DENV. Hence, this study investigates the role of LCs in DENV infection using ex vivo skin model. Isolated migratory LCs (migLCs) or sheets of epidermal human skin (ex-vivo LCs) were exposed to DENV-2 for 48h to determine infection. To research DENV-2 transmission, DENV-2 infected migLCs or ex-vivo LCs were added to DCs for another 48h. Exposure of epidermal skin to DENV-2 ex vivo led to infection of LCs. Furthermore, isolated migLCs were also efficiently infected by DENV-2. Strikingly, both migLCs and ex-vivo LCs transmitted DENV-2 to DCs, leading to high infection of DCs. Interestingly, transmission was dependent on both active DENV-2 infection of LCs and cellular interaction between LCs and DCs. These results demonstrate that LCs are not only permissive for DENV-2 infection, but also play a role in the transmission of DENV-2 to DCs. Normally, LCs fulfill a protective role against invading viruses by degrading viruses such as HIV-1. In contrast, this study provides evidence for a LC-dependent transmission route for DENV-2 infection of DCs and potentially other professional phagocytes. Therefore, preventing infection of LCs might limit dissemination of the virus throughout the host.

P.E1.01 Visualizing immune responses - Part 1

P.E1.01.01

Molecular imaging of retargeted UniCAR T cell therapy

*N. Berndt[1], S. Albert[2], C. Arndt[3], S. Koristka[3], A. Feldmann[3], M. Bachmann[3,4,2], **R. K. Bergmann**[3];*
[1]German Cancer Consortium (DKTK), partner site Dresden and German Cancer Research Center (DKFZ), Heidelberg, Germany, [2]University Cancer Center (UCC) Dresden, Tumor Immunology, 'Carl Gustav Carus' Technische Universität Dresden, Dresden, Germany, [3]Helmholtz-Zentrum Dresden-Rossendorf, Dresden, Germany, [4]German Cancer Consortium (DKTK), partner site Dresden and German Cancer Research Center (DKFZ), Heidelberg, Dresden, Germany.

The switchable UniCAR platform avoiding "off target" side effects by consisting of two components: UniCAR-modified T cells and specific targeting modules (TMs). For personalized precise immunotherapy, molecular imaging regarding *in vivo* localization and kinetics of these components is mandatory. In this presentation, we demonstrate the optical (OI), MRI and PET imaging of this therapeutic concept in a preclinical setting using EGFR positive tumors in mice.UniCAR 28/ζ T cells were labeled with fluorescent nanoparticles and the cell line A431 was transfected with the firefly luciferase. For PET imaging studies both, mono- and bivalent α-EGFR TMs were conjugated with p-SCN-Bn-NODAGA and radiolabeled with ^{64}Cu .The bivalent α-EGFR TM showed an improved redirection of UniCAR T cells against EGFR$^+$carcinoma cells and was able to bind cell lines expressing high to low levels of EGFR. The killing of the tumor cells was visualized and quantified by luminescence imaging. In biodistribution studies both, mono- and bivalent α-EGFR TMs showed specific tumor accumulation after short times and tumor to muscle ratios of more than 12, however, at the next day the tumor to background ratio of the bivalent α-EGFR was higher. The T cells were transiently accumulated in the lungs before they were distributed in the body and also accumulated in lymph nodes. The study shows, that preclinical molecular imaging of all components of the retargeted UniCAR T cell therapy is possible and has the potential to be translated into the clinic.

P.E1.01.02

Neutrophil migration via lymphatic vessels in *Aspergillus fumigatus*-induced inflammation

*M. Shevchenko[1], **A. Fedorina**[1], E. Bolkhovitina[1], A. Bogorodskiy[2], V. Borshchevskiy[2], A. Sapozhnikov[1];*
[1]Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russian Federation, [2]Moscow Institute of Physics and Technology, Dolgoprudny, Moscow region, Russian Federation.

Neutrophils migrate fast from bone marrow to the site of inflammation. Recent studies have demonstrated that neutrophils can also relocate from the site of inflammation toward lymph nodes via lymphatic vessels. Here we investigate the process of neutrophil migration during *A. fumigatus* infection.
For the research we used three-dimensional visualization of whole-mount conducting airways of mice at different time points after *A. fumigatus* inhalation. Tissue samples were stained against Ly6G, Lyve-1 and CD31. Simultaneous visualization of neutrophils, lymphatic vessels and blood vessels was achieved by spectral unmixing.
The optimal sets of primary and secondary antibodies were chosen and simultaneous staining of neutrophils with rat anti-Ly6G, blood vessels with goat anti-CD31 and lymphatic vessels with rabbit anti-Lyve-1 in combination with secondary donkey anti-rat-Alexa488, donkey anti-goat-Alexa555 and donkey anti-rabbit-Alexa647 was performed. We visualized neutrophils that were associated with blood vessels in 6 hours after *A. fumigatus* conidia application. Examination of mouse bronchus in 72 hours after conidia inhalation revealed neutrophil association with lymphatic vessels.
Thus, neutrophils were detected in lymphatic vessels at the late stage of *A. fumigatus* conidia application-induced immune response. The observation confirms the ability of neutrophils to migrate from the site of inflammation via lymphatic vessels.
The study was supported by RFBR № 18-315-00166

P.E1.01.03

Time course of different apoptotic stages during target cell killing

***K. S. Friedmann**, A. Knörck, S. Zöphel, S. Renno, C. Hoxha, M. Hoth, E. C. Schwarz, C. Kummerow;*
Biophysics, CIPMM, Homburg, Germany.

Introduction: CD8$^+$ T- and NK cells are key players for elimination of cancer cells. Killing of such target cells can be induced by two pathways: either perforin-dependent via lytic granules or receptor mediated via Fas-FasL-interaction. Both pathways can lead to apoptosis, whereas only perforin can induce direct target cell lysis (necrosis). The apoptotic pathway involves the activation of caspases leading to cytoskeletal breakdown, DNA fragmentation and loss of membrane integrity. Nevertheless the kinetic progression of the different apoptotic stages during target cell killing by immune effector cells is not well understood.
Methods: We investigated apoptosis induction by primary NK cells and melanoma-specific CD8$^+$ T-cell clones in K562 and Jurkat T-cells or T2 and melanoma cells respectively. We analyzed caspase activation and membrane disruption using amongst others the FRET-based cell death sensor Casper3-GR and the phosphatidylserine sensor AnnexinV. Experiments were performed using flow cytometry and fluorescent live cell imaging.
Results: We describe different apoptotic stages induced by either chemical substances or lymphocyte-mediated killing. As expected, detection of caspase activation is an early apoptotic event. AnnexinV, commonly used as apoptosis marker, on the other hand provides signals which appear in rather late stages of targeted cell death.
Conclusion: The detection of caspase activity during target cell killing to analyze apoptosis is an earlier marker compared to AnnexinV. Moreover the cell death sensor Casper3-GR enables a specific analysis of cytotoxic mechanisms by providing information about target cell morphology and a precise discrimination of apoptotic and necrotic killing on single cell level.

P.E1.01.04

Cryoglobulin clearance after direct acting antiviral -DAA- therapy in hepatitis C virus HCV-monoinfected and HIV-HCV coinfected patients

***M. N. Kolopp Sarda**[1,2], K. Hartig-Lavie3, P. Miailhes4, A. Uhrès5, V. Virlogieux3, P. Pradat3, F. Zoulim3;*
[1]CH Lyon Sud Lab Immunology, LYON - Pierre Bénite, France, [2]EA 4130 Immunogenomic and Inflammation, Lyon Claude Bernard University, France, [3]Hepatology Dpt, Croix-Rousse Hospital, LYON, France, [4]Infectious Diseases Dpt, Croix-Rousse Hospital, LYON, France, [5]Pharmacology Dpt, Croix-Rousse Hospital, LYON - Pierre Bénite, France.

Background: Mixed cryoglobulins (MC) are found in 40-60% of patients with chronic HCV infection. The objective of this study was to evaluate the efficacy of DAA therapy on cryoglobulin clearance in patients with HCV-associated MC. Methods: HCV mono-infected and HIV-HCV co-infected patients with symptomatic or nonsymptomatic MC, who received DAA treatment, were included from 2013 to 2016. Cryoglobulins were characterized and quantified in Immunology laboratory. Results: 41 patients were analyzed: 31 HCV mono-infected patients and 10 HIV-HCV co-infected patients. MC was symptomatic in 77.4% of HCV mono-infected patients and in 10% of HIV-HCV co-infected patients. Two patients had Waldenström macroglobulinemia (WM) and 2 rheumatoid arthritis (RA). The most frequent symptoms were arthralgia, asthenia and polyneuropathy. DAA therapy was used with Ribavirin in 10 patients and all other patients received an IFN-free and Ribavirin-free DAA regimen. Median level of cryoglobulin fell from 51.8 mg/L at baseline to 6.5 mg/L at the end of DAA treatment.
Cryoglobulin clearance was obtained in 66% (n=27) of our cohort: 90% in HIV positive and 58% in HCV mono-infected patients. A partial response with a decrease of cryoglobulin level >50% without negativation was reached in 15% of patients. Cryoglobulin was persistent in patients with WM and RA. Baseline cryoglobulin level was not associated with cryoglobulin clearance at the end of treatment. Similarly, the presence of symptomatic MC, fibrosis stage and HCV genotype were not associated with cryoglobulin clearance.
Conclusions: DAA therapy allows a high rate of cryoglobulin clearance both in HCV monoinfected and HIV-HCV coinfected patients.

P.E1.01.06

Predicting NK cell behavior with mathematical models

A. J. Millan[1], *S. Elizaldi*[1], *E. Lee*[1], *J. Aceves*[1], *D. Murugesh*[2], *G. G. Loots*[2], *J. O. Manilay*[1];
[1]*University of California, Merced, Merced, United States,* [2]*Lawrence Livermore National Laboratory, Livermore, United States.*

Natural killer (NK) cells are specialized lymphocytes with innate ability to eliminate virally infected and cancerous cells, but the mechanisms that control NK cell development and cytotoxicity are incompletely understood. We have identified novel roles for sclerostin domain containing-1 (Sostdc1) in NK cell development and function. Sostdc1-knockout (KO) mice display a progressive accumulation of transitional NK cells (CD27+CD11b+, tNK) with age, indicating a partial developmental block. We simulated developmental rates with a deterministic compartmental model under the assumption that proliferation and death rates stay consistent. Our model elucidates a requirement for Sostdc1 in NK cell development. Furthermore, we identified that Sostdc1-KO splenic tNKs express lower frequencies (%) of inhibitory Ly49G2, but higher % of activating Ly49H+ and D+ cells. However, the % of Ly49I+, G2+, H+ and D+ populations were universally decreased at the mature (CD27- CD11b+, mNK) stage. We hypothesized that the Ly49 repertoire and developmental block in Sostdc1-KO mice would correlate with NK killing ability. We observed that KO NK cells are hyporesponsive against MHC-I-deficient cell targets in vitro and in vivo, despite higher CD107a surface levels and similar IFN-gamma expression to controls. We plan to use high-dimensional flow cytometry analysis to identify specific NK cell clusters of highly active subsets based on Ly49 and activating receptor expression. Taken together, these data support a role for Sostdc1 in the regulation of NK cell development and could provide insights into novel biological parameters to increase active NK cell numbers with high killing efficiency for immunotherapies.

P.E1.01.07

Direct measurements of tumor antigen cross-presentation by dendritic cells

E. M. Muntjewerff, *F. Bianchi, G. van den Bogaart;*
Radboud Institute for Molecular Life Sciences, Nijmegen, Netherlands.

Introduction: Antigen cross-presentation is essential for the initiation of an adaptive immune response against cancer and numerous pathogens. During this process, dendritic cells (DCs) present peptides derived from ingested antigens of affected tissues in their major histocompatibility complex class I (MHC-I) to cytotoxic CD8+T cells. As a result, cognate CD8+T cells become activated, enabling them to eliminate affected cells in the human body. Aim of this study is to develop a quantitative method to measure cross-presentation of NY-ESO1, a tumour-specific antigen expressed by several cancer types.
Materials and Methods: HLA-A2+ DCs were activated and loaded with a NY-ESO1-based antigen consisting of a HLA-A2-binding epitope extended at its N- and C-termini. To measure cross-priming, these DCs were co-cultured with primary autologous CD8+T cells. These T cells were transfected with mRNA coding for a T cell receptor specific for the NY-ESO1 epitope. After incubation, the activation of the cytotoxic T cells was followed using ELISA for interferon γ and flow cytometry for T cell activation and proliferation markers. In addition, we also followed the dynamics of bio-orthogonally tagged NY-ESO1 peptides carrying a solvent-exposed propargylglycine, enabling for direct visualization of antigen cross-presentation independent of co-stimulatory signals.
Results & conclusion: This novel method allows to measure presentation of tumour antigens on MHC-I in dendritic cells. Since cross-presentation is crucial for elimination of virus-infected and malignant cells by the immune system, this knowledge could contribute to improvement of DC-based immune therapies.

P.E1.01.08

DNA methylation profiles identify epigenetically regulated host factors associated with immune control of HIV infection

B. Oriol-Tordera[1], *M. Berdasco*[2], *A. Llano*[1], *B. Mothe*[1,3], *C. Galvez*[1], *J. Martinez-Picado*[1,3,4], *C. Ganoza*[5], *B. Clotet*[1,3], *M. L. Calle*[3], *A. Sanchez-Pla*[6,7], *J. Sanchez*[5,8,9], *M. Esteller*[2,4,10], *C. Brander*[1,4,11], *M. Ruiz-Riol*[1];
[1]*IrsiCaixa, AIDS Research Institute, Hospital Germans Trias i Pujol, Institute for Health Science Research Germans Trias i Pujol (IGTP), Badalona, Spain,* [2]*Cancer Epigenetics and Biology Program (PEBC), Bellvitge Biomedical Research Institute, L'Hospitalet de Llobregat, Barcelona, Spain, Hospitalet de Llobregat, Spain,* [3]*University of Vic - Central University of Catalonia, Vic, Spain,* [4]*Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain,* [5]*Asociación Civil IMPACTA Salud y Educacion, Lima, Peru,* [6]*Statistics Department, Biology Faculty, University of Barcelona, Barcelona, Spain,* [7]*Statistics and Bioinformatics Unit Vall d'Hebron Institut de Recerca [VHIR], Barcelona, Spain,* [8]*Department of Global Health, University of Washington, Seattle, United States,* [9]*Centro de Investigaciones Tecnológicas, Biomédicas y Medioambientales, Lima, Peru,* [10]*Department of Physiological Sciences II, School of Medicine, University of Barcelona, Barcelona, Spain,* [11]*Aelix Therapeutics, Barcelona, Spain.*

The epigenetic regulation of host factors associated with in-vivo control of HIV has been little explored. To start addressing some of the gaps in our knowledge in this regard we assessed host genome methylation signatures in the peripheral blood mononuclear cells (PBMC) of untreated chronically HIV-infected subjects with high (>50000 HIVcopies/ml, n=29) or low HIV (<10000 HIVcopies/ml, n=42) plasma viral loads (VL). Differential methylated CpG sites between the two groups were determined using regression models, adjusting for methylation confounders (age, sex, PBMC cell type composition). Clustering and gene enrichment analysis were applied to identify signatures of antiviral genes putatively epigenetically regulated and which were positively (e.g.CXCR5) or negatively (e.g.MX1) correlated with viral load (Rho>0.6) and proviral reservoir (Rho>0.5) in total PBMC. Based on methylation profiles, the gene expression levels of lead candidates, including MX1, a strong antagonist of DNA- and RNA-virus replication, were determined by real-time PCR. MX1 was significantly higher expressed in individuals with elevated HIV viral load compared to low-viral load subjects. Additionally, MX1 gene expression and promoter methylation levels were inversely correlated (Rho=-0.55), further supporting a tight epigenetic regulation of the expression of this gene. Additional MX1 gene expression analyses in unrelated cohorts of individuals with chronic uncontrolled HIV infection (n=11) and HIV elite controllers (VL<50 HIVcopies/ml, n=12) validated these results. The data thus identify several epigenetically dysregulated antiviral factors, MX1 in particular, that may be employed in future immune interventions aiming at the cure of HIV infection.

P.E1.01.09

Natural Killer cells switch from Granzyme B to death receptor-mediated cytotoxicity during serial killing of tumor cells

I. Prager[1], *C. Liesche*[2], *H. van Ooijen*[3], *D. Urlaub*[1], *M. Claus*[1], *B. Önfelt*[3,4], *J. Beaudouin*[2], *C. Watzl*[1];
[1]*Leibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany,* [2]*Division of Theoretical Bioinformatics, German Cancer Research Center, Heidelberg, Germany,* [3]*Department of Applied Physics, Science for Life Lab, KTH – Royal Institute of Technology, Stockholm, Sweden,* [4]*Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden.*

Natural Killer (NK) cell cytotoxicity is mediated via the release of cytotoxic granules or by engaging death receptors that initiate caspase cascades. While the mechanisms of the two pathways are well understood, the orchestrated interplay between both has remained poorly defined. To visualize the granzyme B (GrzB) and Caspase-8 mediated target cell death in a time-dependent manner, we used fluorescent localization reporters that enabled us to simultaneously measure the activities of both pathways upon contact with NK cells by life cell imaging.We observed rapid target cell death which was induced by GrzB and which originated from early established NK : target contacts. In contrast, cell death mediated by Caspase-8 was a result of later target cell engagements and took much longer from contact to target cell death. We determined the amount of intracellular GrzB, perforin and of surface-FasL in NK cells that were exposed to target cells over time.
We observed a clear reduction of GrzB and perforin and an increase of surface-FasL in NK cells over time. This suggested a kinetic regulation of the two cytotoxic pathways during serial killing. By directly imaging individual NK cells during serial killing we could indeed show that NK cells switch from mediating a fast GrzB-mediated cell death in their first killing events to a more delayed death receptor-mediated cell death during subsequent tumor cell encounters. This demonstrates that the use of GrzB vs. death receptor-mediated target cell killing is differentially regulated during the serial killing activity of NK cells.

P.E1.01.10

Expression of equine interleukin (IL)-2, IL-4, IL-10, IL-18 and IFN-γ in Baculovirus expression system

S. Saini[1,2], *H. Singha*[1], *P. Siwach*[2], *B. N. Tripathi*[1];
[1]*National Research Centre on Equines, Hisar, India,* [2]*Chaudhary Devi Lal University, Department of Biotechnology, Sirsa, India.*

Recombinant cytokines are considered as valuable tools for functional studies and candidates for vaccine additives or therapeutic use in various diseases. In the present report, horse IL-2, IL-4, IL-10, IL-18 and IFN-γ were produced in insect cells using baculovirus expression system. The coding sequences of equine cytokines were cloned in gateway entry vector (pENTR™/TEV/D-TOPO vector). Subsequently, recombinant cytokines were mobilized from entry vector to baculoviral destination vector DNA (Baculodirect ™ linear DNA). Further, *Spodoptera frugiperda* (SF9) cells were transfected by baculovirus/respective cytokine ligation mix and incubated at 27^0C. Multiplicity of infection (MOI) ranging from 1-10 PFU and optimum expression time was standardized for each cytokine. After 72 h post-infection, reduced cell growth, enlargement of cell nuclei, polyhedron formation and partially detached cells were observed. Expression of recombinant horse cytokines in SF9 cells was confirmed by SDS-PAGE and western blotting. Recombinant baculovirus DNA was confirmed by PCR and sequencing cytokine insert. Recombinant horse cytokines were purified from cell pellet by using Nickel-NTA affinity chromatography. SDS-PAGE analysis displayed the molecular weight of IL-2, IL-4, IL-10, IL-18 and IFN-γ at 22, 18, 24, 23 and 24 kilo dalton (kDa), respectively. These recombinant horse cytokines will provide basic research tool for understanding their role in protective immune response, disease pathogenesis, and use as a therapeutic agent or vaccine adjuvant in horses.

P.E1.01.11

Full automated load-and-go flow cytometry in neutrophil analysis: the start of a new era

***R. Spijkerman**, L. Hesselink, P. Hellebrekers, F. Hietbrink, L. Leenen, L. Koenderman;*
UMC Utrecht, Utrecht, Netherlands.

Introduction Changes in functional phenotypes of neutrophils is an adequate measurement of the amplitude of the innate immune response. A combination of these changes can be applied to predict infectious complications. Due to technical and logistical difficulties this concept is currently not clinically applicable. A fully automated 24/7 load and go flow cytometer would provide the opportunity to design such an analysis. Therefore, the aim of this study was to investigate the applicability of 24/7 automated analysis of neutrophils by flowcytometry. **Methods** For proof of principle blood was drawn from healthy controls next to the flow-cytometer. Neutrophil activation was measured by the use of the automated AQUIOS load-and-go flowcytometer. The AQUIOS is able to pierce the tube caps, add antibodies, lyse and measure the sample within 20 minutes immediately after vena puncture. Thereafter, the *same* blood tubes were measured every 15 minutes. Measurements were done in presence or absence of the bacterial stimulus fMLF. **Results** A significant increase in MFI was detected in the activation markers CD35(174%(146%-213%),**P=0.004**), CD11b(245%(167%-524%),**P=0.009**) and CD11c(220%(153%-389%),**P=0.008**) within the first hour after vena puncture. After 3 hours an even higher increase in all activation markers was detected (CD35(222%);CD11b(378%);CD11c(316%)). Neutrophil responsiveness to fMLF was most evident at T=0 and gradually decreased over time. **Conclusion** Neutrophil activation significantly increased in the tube shortly after vena puncture particularly during the first hour. For a reproducible clinical test on neutrophil functionality it is mandatory to measure neutrophil receptor markers immediately after vena puncture in a point-of-care context.

P.E1.01.12

Identification of germline heavy chain targeted to envelope protein in acute phases of DENV2 secondary infected patients.

***N. Thammasonthijarern**;*
Tropical Medicine, Bangkok, Thailand.

Identification of germline heavy chain targeted to envelope protein in acute phases of DENV2 secondary infected patients.

N. Thammasonticharoen[1], W. Poungmanee[1], S. Benjathummarak[1], P. Ramasoota[1], P. Pitaksajjakul[1]
[1] Department of Social and Environmental Medicine, Faculty of Tropical Medicine, Mahidol University, Thailand.

Dengue is one of the most important mosquito-borne viral diseases in humans, and lacking of vaccines and therapeutics contributes to disease burden. Dengue viral infection can cause a spectrum of clinical symptoms ranges. The understanding of human immunity creating antibody response that showed protection against infectious agent can be applied for vaccine design strategy.

Antibody heavy chain genes generated at acute phase of secondary DENV-2 infected patients were studied from both human monoclonal antibodies (HuMAbs) targeted to enveloped (E) proteins by clonal sequencing, and sample of B cell repertoires by next-generation sequencing. In addition, antibody neutralizing activity was tested by foci reduction neutralization test (FRNT). Germline sequencing were analyzed either by IMGT/V-Quest or IMGT/HighV-Quest online program.

Two major germline genes derived from 25 cross-neutralizing HuMAbs were IGHV1-69 and IGHV3-23. Accordingly, IGHV1-69 is also the majority (32%) of antibody heavy chain genes derived from PBMC of acute phase dengue type 2 infected patient. It was implied that IGHV1-69 type of heavy chain was rapidly generated after DENV-2 infection at the acute phase and showed cross-neutralizing activity to 4 serotype and showed cross-protection in dengue patients. The result obtained from this study will be understanding for human antibody response correlated with further dengue vaccine design.

KEY WORDS: Dengue, PBMC, HuMAbs,

P.E1.01.14

Identification of small molecule compounds modulating the formation of neutrophil extracellular traps (NETs)

*P. Habenberger[1], **P. Wentker**[1], R. Di Lucrezia[1], G. Sollberger[2], M. Bickle[3], J. Eickhoff[3], A. Zychlinsky[2], P. Nussbaumer[1], A. Choidas[1], B. M. Klebl[1];*
[1]Lead Discovery Center GmbH, Dortmund, Germany, [2]Max Planck Institute for Infection Biology, Berlin, Germany, [3]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

Formation of neutrophil extracellular traps (NETs) is a non-reversible process of subsequently appearing, distinct cellular morphological features, which includes the loss of the characteristic lobulated nucleus. Breakdown of the nuclear and cellular membranes finally leads to release of several granule proteins and DNA / chromatin forming the extracellular NET fibres. Normally functioning as defence mechanism against extracellular pathogens, NET formation was also shown to be involved in the pathogenesis of several infectious diseases, sepsis, chronic lung disease, thrombosis and autoimmune disorders (e.g. systemic lupus erythematodes). Aiming at potential therapeutic applications, we performed a high throughput screen on the basis of those neutrophilic morphological changes to identify small molecular weight inhibitors of NET formation.

Primary human neutrophils from healthy donors were stimulated with phorbol 12-myristate 13-acetate (PMA) and treated with ~180.000 compounds. After staining the DNA for fluorescence microscopy, high content analysis was used to determine the NET-related morphological changes. This phenotypic screen identified hits in several compound classes. The assay quality parameters within the screening campaign were good, especially for an assay using human primary cells. Upon hit verification, the results were clustered chemically and biologically into different groups. In parallel to hit optimization, selected compounds have been further characterized to unravel their mode of action.

In summary, we identified compounds modulating the formation of NETs by a high content screen based on phenotypic morphological changes in human primary neutrophils. Our results serve as basis of further investigation of immunomodulatory drugs and promote an innovative drug discovery program.

P.E1.02 Visualizing immune responses - Part 2

P.E1.02.01

ACUTE AND CHRONIC INFLAMMATION AND MAST CELL ACTIVATION, IN SILICO

***A. Abdukerim**;*
VU University Amsterdam, Amsterdam, Netherlands.

Objective: An iterative process of model building is able to provide new understanding about the mechanisms of inflammation. We designed a computer model able to provide a rational explanation of the network's response to antigen in terms of acute or chronic inflammation. The model should calculate *in silico* the level of tumor necrosis factor alpha (TNF-α). It should likewise predict the effect of an *in silico* drug targeting the immunoglobulin light chain (FLC) that activates mast cells, on the outcome of an inflammation. **Modelling:** For each species in the network, a balance equation was then formulated, specifying its time dependence as the differences between the rates of the reactions synthesizing the commodity and the rates of the processes degrading it. The corresponding rate equations were integrated in time or for steady state by using the COPASI simulation software. Immunostaining on cancer biopsies, protein detection, and *in vitro* and *in vivo* studies were used for validation. **Results:** The model enabled us to 1) examine the capability of immune system to kill invading microorganisms, 2) explain the dichotomy between acute and chronic inflammation, and 3) test effect of an in silico peptidic drug that inhibits FLC. The model reproduced experimental findings and was subjected to tests for dynamic stability and to sensitivity analysis. **Conclusions:** A systems mechanism for the effects of therapeutic peptides against cancer-associated inflammation was identified and made computable in terms of personalized molecular properties. This should facilitate further testing as well as provide a platform for personalized immunology.

P.E1.02.02

Tetracysteine tagged $OVA_{(323-339)}$peptide as a novel tool for visualizing peptide-MHCII complexes in primary mouse cells

***B. Akkaya**, J. Al Souz, R. Kamdar, O. Kamenyeva, E. M. Shevach, M. Akkaya;*
NIH, Bethesda, United States.

Current reagents such as peptide specific MHCII antibodies do not allow to probe peptide-MHCII dynamics during live interactions in vivo as they also block T cell- APC interactions. To address this, we aimed to design fluorescent antigenic peptides. Typically, flurorescent protein tags such as green fluorescent protein (GFP) consist of more than 200 amino acids thus have the potential of disrupting the conformation and functionality of the antigenic peptides. Therefore, we constructed an $OVA_{(323-339)}$ peptide containing a pro-fluorescent tetracysteine tag (CCPGCC) at the C-terminus with aminocaproic acid linker (OVA_{CACA}). First, we tested whether the addition of tag had an impact on T cell stimulation and found that OVA_{CACA} pulsed APCs could activate OTII cells to same degree as $OVA_{(323-339)}$. Secondly, we treated the APCs with an organoarsenic compound to switch on the fluorescence and measured the signal by flow cytometry. We showed that the fluorescent signal specifically comes from OVA_{CACA}-MHCII as neither $MHCII^{-/-}$ APCs that were pulsed with OVA_{CACA} nor $MHCII^{+/+}$ APCs that present $OVA_{(323-339)}$ produced same fluorescence. We also tracked the fluorescence in OTII- DC culture in vitro and detected that it got concentrated at the synapse over time. Altogether our findings suggest that tetracysteine tagged peptides can be used for tracking the peptide-MHCII complexes during visualizing the interactions of primary mouse T cells and APCs and they have the potential to be an alternative to MHCII tetramers to detect antigen specific T cells.

P.E1.02.03

Infiltrating T cells establish immunological synapses forming cSMAC preferentially with MHCII antigen presenting cells in human GBM.

***C. Barcia**, L. R. Diaz, E. Saavedra-Lopez, G. P. Cribaro, P. Casanova, L. Romarate;*
Institut de Neurociències, Bellaterra, Cerdanyola del Vallès, Spain.

Glioblastoma multiforme (GBM) is the highest and most aggressive grade of glioma, the most common primary brain tumor. Currently it remains incurable, as there are no effective treatments; however, the presence of an inflammatory environment characterized by immune cell infiltration and microglial activation, has suggested that its manipulation could represent a potential therapeutic strategy. In this study, immunological engagements between infiltrating T cells with tumor cells and microglia/macrophages were analyzed in detail. Our results show that 70% of T cells establish physical appositions with either tumor cells or MHCII+ cells, and that 55% of contacting T cells form features of immunological synapses, being much more likely to do so with MHCII+ cells, since the number of TCR rich central supramolecular activation clusters (cSMACs) formed with these cells doubled the cSMACs formed with tumor cells. Importantly, we demonstrate that antigen presentation mediated by MHCII takes place in the tumor parenchyma and not only in the lymph nodes, suggesting that the brain tumor could be acting as a tertiary antigen presenting tissue to downregulate the cytolytic immune response. Furthermore, we believe that the IS formation between T cells and MHCII cells may be an strategy of the tumor to escape the immune response (i.e activating antigen-specific Tregs), so the inhibition of these engagements could represent a new immune checkpoint to block for potential immunotherapy in GBM.

P.E1.02.04

Real-time migratory pattern monitoring of neutrophil and macrophage toward skin melanoma microenvironment using two-photon intravital microscopy

***Y. Choe**, S. Jeong, Y. Kim, Y. Hyun;*
Department of Anatomy and Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea, Republic of.

<META NAME="author" CONTENT="최영호(해부학교실)">
Recent studies revealed that recruitment of myeloid cells toward tumor microenvironment can accelerate tumor progression during tumor development. However, the initial response of neutrophils and macrophages interacting with tumor cells in early phase has not been clearly visualized.
Hereby, we investigated the crosstalk between metastatic skin melanoma and innate immune cells including neutrophils and macrophages. To directly visualize migratory pattern of neutrophils and macrophages toward melanoma microenvironment, we set up dorsal skin window chamber for two-photon intravital microscopy. In order to investigate dynamic behavior, CMFDA (green) or CMTPX (red) fluorescent probe-labeled murine melanoma cells, B16F10, were subcutaneously injected. To visualize neutrophils and macrophages, we used LysM-GFP and CX3Cr-1 GFP mice, respectively, as well as fluorescent Abs.
In this study, we will visualize each step of neutrophil infiltration toward melanoma cells at the early stage of subcutaneous injection of B16F10, and also demonstrate how to control the adhesion molecule developmental endothelial locus-1 (Del-1) affects the migration pattern of neutrophils. We hypothesized that Del-1 deficiency may be involved in the intravascular and transendothelial migration of neutrophils in the presence of B16F10 melanoma cells. However, it was supposed that absence of Del-1 may not affect the interstitial migration of neutrophil to the tumor site. In this study, we will show the effect of Del-1 in neutrophil migratory cascade toward to melanoma microenvironment in crosstalk with resident macrophages in the early phase of innate immune response.

P.E1.02.05

A comparative analysis of targeted transcriptomic techniques and immunohistochemistry for immuno-oncology biomarker profiling in humanized mice

***M. Houtkamp**, N. Pencheva, F. de Bree, P. Franken, S. Verploegen, D. Schuurhuis, J. Lammerts van Bueren;*
Genmab BV, Utrecht, Netherlands.

Traditional transcriptomic profiling techniques, such as RNA sequencing and microarrays, require relatively large sample input and their application on formalin-fixated paraffin-embedded (FFPE) samples is often hindered by RNA degradation. NanoString and HTG EdgeSeq are novel technologies that measure messenger RNA (mRNA) levels in a targeted fashion and can be applied to low amounts of FFPE samples for biomarker discovery. The ability of these targeted transcriptomic approaches to capture immune-related changes, as validated by immunohistochemistry (IHC), was assessed in human hematopoietic stem cell-reconstituted mice containing subcutaneous human tumors treated with a T-cell activating agent versus control. In addition, the NanoString PanCancer Immune Profiling Panel and the HTG immuno-oncology (IO) assay were compared for their concordance. Strong concordance (r=0.8, Pearson) between the NanoString and HTG techniques of mRNA measurements were observed. Both techniques identified a largely overlapping set of transcripts modulated upon treatment with T-cell activating agent. Importantly, both NanoString and HTG EdgeSeq mRNA measurements were further corroborated by quantitative IHC protein measurements for a selected panel of IO biomarkers modulated by T-cell activating treatment. In conclusion, both NanoString and HTG EdgeSeq are suitable technologies for transcriptomic assessment of IO biomarkers in FFPE samples. T-cell activating agent-induced transcriptomic changes, as assessed by either technology, are representative of immune alterations found by IHC - the current gold standard in the IO field.

P.E1.02.06

Identification of apoptotic cells and cells carrying extracellular vesicles during LCMV infection using imaging flow cytometry

***J. Kranich**[1], N. Chlis[2], A. Latha[1], L. Rausch[1], F. Theis[2], T. Brocker[1];*
[1]Institute for Immunology, 82152 Planegg-Martinsried, Germany, [2]Helmholtz Zentrum München, 85764 Neuherberg, Germany.

Exposure of the phospholipid phosphatidylserine (PS) on the outer surface of the cell membrane is common to both, apoptotic cells and extracellular vesicles (EVs). Administration of a fluorescent version of the PS-binding protein Mfge8 (Mfge8-eGFP) *in vivo* in combination with imaging flow cytometry, allowed us to reliably detect and separate dying cells from live EV-decorated cells *ex vivo*. To discriminate both classes of Mfge8-eGFP$^+$ cells we developed a deep learning algorithm. Using this novel approach, we found only very low frequencies of dying cells in absence of infection, but EV-decoration was readily detected mainly on B cells. Upon LCMV-infection, however, we found a significant increase in cell death mainly among CD8$^+$ T_{CM} (CD44$^+$CD62L$^+$) cells as well as follicular and marginal zone B cells. In addition, the frequency of EV-decorated CD8$^+$cells increased approx. 4-fold. Here, EV-binding was found to be mostly confined on CD8$^+$ T_{CM} and T_E (CD44$^+$CD62L$^-$), but almost absent on T_N (CD44$^-$CD62L$^+$). Our data visualize and quantify the propensity of activated CD8$^+$T cells to bind EVs, opening new avenues for investigation of potential functional consequences. Our data demonstrate that Mfge8-eGFP is a valuable tool for simultaneous identification of dying and EV-decorated cells *in situ*.

P.E1.02.07

Quantifying murine myocardial infarction and immune cell infiltration via light sheet fluorescence microscopy

***S. F. Merz**[1], S. Korste[2], L. Bornemann[1], P. Stock[2], U. Hendgen-Cotta[2], T. Rassaf[2], M. Gunzer[1], M. Totzeck[2];*
[1]Institute for experimental Immunology and Imaging, University Duisburg-Essen, Essen, Germany, [2]West German Heart & Vascular Center, Department of Cardiology & Vascular Medicine, University Hospital Essen, Essen, Germany.

Myocardial infarction (MI) is one of the most lethal diseases in western industrialized countries. To investigate the underlying mechanisms and to determine effects of novel therapeutic approaches an accurate quantification of infarct sizes in experimental models is imperative. Current protocols utilize life imaging (MRT, echocardiography) or slice-based histological methods, which suffer from poor resolution or limited representation of the entire heart, respectively.
Here, we present a novel light sheet fluorescence microscopy (LSFM) approach for the precise quantification of relevant infarction model parameters including MI-size, area at risk (AAR) and heart volume in adult mice. Benefitting from ethyl cinnamate (ECi) clearing, it allows simultaneous visualization of immune cell infiltrates in the whole heart at cellular resolution via *in vivo* labeling of target structures utilizing i.v. injected conjugated antibodies and fluorochrome compatible bleaching.
Verifying its usefulness, we correlated our LSFM method with traditional TTC staining in an ischemia/reperfusion-injury mouse model. Here, TTC negative areas matched with capillary damage revealed by anti-CD31 labeling of the endothelium in the same heart slices. Furthermore, we visualized macrophages and neutrophils via antibodies against F4/80 and Ly-6G, respectively. Neutrophils (24h reperfusion) were located in the vicinity, while macrophages (5d reperfusion) were localized within the infarction volume.
Taken together, LSFM-based 3D quantification of infarction size represents a novel and precise tool to measure MI and to obtain crucial information about immune cell infiltration at the same time. This powerful approach enables multiplexing of various markers of interest to investigate myocardial damage, infiltrating immune cells and their behavior.

P.E1.02.08

High throughput sequencing of TCR repertoire after yellow fever revaccination

***A. A. Minervina**, M. V. Pogorelyy, E. A. Komech, I. Z. Mamedov, Y. B. Lebedev;*
Shemyakin–Ovchinnikov Institute of bioorganic chemistry, Moscow, Russian Federation.

Introduction: Yellow fever vaccination is a well established model of acute viral infection in human. Primary immunization elicits strong T-cell response and the formation of long-lived memory. However, little data exists on the activation of this memory and individual T-cell clones dynamics after revaccination. We applied deep TCR-profiling to quantitatively track T-cell clones after yellow fever vaccination and revaccination.
Methods: We isolated PBMCs from two donors -- one received primary vaccination, other revaccinated 30 years after -- on 5 timepoints: 0, 5, 10, 15, 45 days after YFV17D immunization. CD4+, CD8+, memory and MHC-dextramer positive subpopulations were collected on several timepoints. TCR repertoires were sequenced on Illumina platform, expanded TCR clonotypes were identified using edgeR package.

Results: We identified 1374 expanded clonotypes in first-time vaccinee and 125 in revaccinated donor. YFV17D-specificity was confirmed by TCR repertoire sequencing of MHC-dextramer positive subpopulation. In first-time vaccinee fraction of responded clones sharply peaked (6%) at day 15 after immunization. However, in revaccinated donor fraction of responded clones reached 0.3% by day 5 and slightly increased on days 10 and 15. Analysis of individual clones dynamics showed presence of two distinct groups of clones in revaccinated donor: peaking on day 5 and on day 15.
Conclusions: Second vaccination with YFV17D-vaccine, even 30 years after first, elicits T-cell response. This response differs from response to primary immunization both by intensity and dynamics. We detected early expansion of memory T-cells in response to revaccination and delayed response of putatively naive T-cells. Supported by RSF-15-15-00178 .

P.E1.02.09
Visualizing tumor cell-lymphocyte interactions in the brain using *in vivo* two photon microscopy

***M. Piechutta**, M. A. Karreman, A. S. Berghoff, K. Gunkel, W. Wick, F. Winkler;*
Neurology Clinic and National Center for Tumor Diseases, University Hospital Heidelberg; Clinical Cooperation Unit Neurooncology, German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany.

Current knowledge on the immunological tumor microenvironment is gained from *in vitro*- and *ex vivo* experiments, such as flow cytometry and immunohistochemistry. However, these models fail to recapitulate the dynamic influx of lymphocytes and their interaction with tumor cells in the complex tumor microenvironment. Particularly in brain cancer, which develops in an "immune-privileged organ", a better understanding of lymphocyte recruitment, trafficking and activation *in vivo* is urgently needed to improve immunotherapeutic approaches. To characterize the adaptive immune response in brain tumors on a single cell level, we developed a unique model allowing us to monitor these processes *in vivo* over weeks using repetitive two photon intravital microscopy. Hereto, mice with a chronic cranial window were introduced with fluorescent syngeneic melanoma cells via heart injection or stereotactic injection into the cortex. Next, these mice received adoptive cell transfer of tumor-specific, fluorescently labeled T cells. We observed tumor-specific T cells crossed the brain blood barrier within a couple of hours. Notably, transferred T cells were found in the tumor margin and inside the tumor but not in healthy brain parenchyma. Furthermore, we could track lymphocytes at the site of the tumor for several days to explore their interactions with tumor cells.
Taken together, we established a novel preclinical model to study dynamic lymphocyte- tumor cell interaction *in vivo* at high resolution. This approach provides unique insights into the underlying mechanisms driving the immuno-response. Importantly, it enables to further investigate and improve immunotherapeutic approaches with respect to prevention and therapy of brain metastases.

P.E1.02.10
Alteration of TCR repertoire after tick-borne encephalitis infection in murine model

***M. A. Salnikova**[1,2], A. A. Minervina[1], M. V. Pogorelyy[1], K. K. Tuchinskaya[3], G. G. Karganova[3], Y. B. Lebedev[1], I. Z. Mamedov[1];*
[1]Shemyakin-Ovchinnikov Institute of Bioorganic chemistry, Moscow, Russian Federation, [2]Moscow State University, Moscow, Russian Federation, [3]Chumakov Institute of Poliomyelitis and Viral Encephalitides, Moscow, Russian Federation.

Introduction: Adaptive immune system is a very sophisticated mechanism, which is able to recognize great diversity of antigens. T cells play key role in adaptive immune response to tick-borne encephalitis virus. Deep TCR repertoire profiling can be performed via high-throughput sequencing. Murine models to study alterations in TCR repertoires during immune response are lacking. Here we present the method for longitudinal TCR repertoire reconstruction which identifies TCR clonotypes expanded in response to acute infection.
Materials and Methods: Peripheral murine blood was collected in two biological replicates before and after the infection with tick-borne encephalitis virus. TCRbeta cDNA libraries were prepared using custom protocol. Libraries were sequenced on Illumina platform. TCR repertoires were extracted from data using MIGEC and MiXCR software.
Results: We show good reproducibility of individual clones concentrations between biological replicates. edgeR package was used to identify significantly expanded or contracted after infection. We found 38 clones expanded and 1 contracted in response to viral infection. Most expanded clonotypes were not detected before infection, but were very abundant after, accounting for 6% of repertoire with 13 responding clones in top 20 clonotypes after the infection. Identified expanded clonotypes show considerable V-usage bias and little overlap with published tick-borne encephalitis virus specific TCRbeta sequences.
Conclusion: This approach can be used to identify statistically significant changes in murine TCR repertoire during infections, vaccinations and drug tests.
Supported by RSF15-15-00178.

P.E1.02.11
In vivo deuterium labeling of circulating immune cells: a feasibility study

***E. Veld, in 't**[1], H. W. Grievink[1], M. Baliu Pique[2], J. Drylewicz[2], F. E. Stuurman[1], T. Kolk, van der[1], G. J. Groeneveld[1], J. A. Borghans[2], K. Tesselaar[2], M. Moerland[1];*
[1]Centre for Human Drug Research, Leiden, Netherlands, [2]UMCU, Utrecht, Netherlands.

Quantification of the life span of immune cells *in vivo* by deuterium labeling may be an attractive methodology when studying disease or drug effects. It requires cell isolation, which is operationally challenging in clinical studies. Moreover, limited data are available on the variability of cellular lifespan between subjects, and the correlation with cell counts. Therefore, a feasibility study was performed in 16 volunteers (8 healthy subjects/HV, and 8 MS patients). Subjects received 70% deuterated water for 9 weeks and were followed for one year. Immune cell subsets were isolated by sequential magnetic sorting (RoboSep), phenotyped (MACSQuant10), and analyzed for deuterium incorporation (GCMS) allowing calculation of life span.
Cell count strongly influenced cell purity. For CD19+ B-cells and CD4+ T-cells, average purities were 89 and 87% (CD4+, HV and MS) and 90 and 89% (CD19+, HV and MS). Cell counts were generally exceeding $30*10^4$/subset. However, for memory and naïve CD8+ T-cells, average purities were 76 and 59% (CD8+m, HV and MS) and 73 and 68% (CD8+n, HV and MS), with counts below $15*10^4$. Average life span was 271±232 days for CD19+ cells, and 209±283 days for CD4+ cells (minimal purity 85%), not clearly correlating with circulating cell numbers.
Although the availability of automated sorters greatly facilitates cell sorting in clinical studies, our data demonstrate that the quality of the cells remains an important point-of-attention for customized isolation protocols. Life span of immune cell subsets was highly variable between subjects, complicating its use as readout in future clinical pharmacology studies.

P.E1.02.12
In vitro three dimensional vascularized skin on a chip to study of immune response

***S. Kim**;*
KIST, Seoul, Korea, Republic of.

Various kinds of human organ on a chip studies have been performed to screen the toxicity and efficacy of certain materials including drugs by various limitations of animal experiments such as the ethical and regulatory issues and the considerable difference between animals and human. Especially, skin on a chip is one of the studies that has attracted attention because it can be used for screening of cosmetics and skin detergents as well as drugs. However, most of current in vitro skin models are based on two dimensional culture of fibroblasts and keratinocytes that only simulate human epidermis and dermis. One of the most serious problems among various skin reactions caused by chemical substances and biological agents, including drugs, cosmetics and skin detergents is inflammatory skin disease. To confirm this reaction, the vascular network structures, which regulate immune response, are need as well as the epidermis and dermis structures. For this reason, we investigated the in vitro three dimensional vascularized skin on a chip to mimic the structures and functional responses of the human skin using collagen/fibrin hydrogel (CFH) for dermis and poly(l-lactide-co-caprolactone)(PLCL) nanofibrous electrospun membrane(NEM) for epidermis. To mimic vessels, we created channels in the CFH and coated with human umbilical vein endothelial cells(HUVECs). Consequently, we could find that channels coated with HUVECs formed a vessels-like structure, and microvascular networks were formed in CFH like the dermis. Moreover, it was confirmed that PLCL-NEM formed the epidermis like structure.

P.E1.02.13
Characterization of immune infiltrate and checkpoint protein expression patterns in murine syngeneic tumors via multiplex immunohistochemistry

***J. Bewsher**[1], J. Ziello[2], S. Klein[2], K. Costa[2], K. Crosby[2];*
[1]Cell Signaling Technology, Leiden, Netherlands, [2]Cell Signaling Technology, Danvers, United States.

Murine syngeneic tumor models are increasingly utilized for preclinical immuno-oncology studies as immunotherapeutic strategies continue to make clinical strides. However, the immunologic features of the tumor microenvironment (TME) in these models remain largely undefined. In this study, we applied a 7-color multiplex immunohistochemistry (mIHC) panel to visualize and quantify the immune infiltrate within formalin-fixed, paraffin-embedded (FFPE) Renca, CT26, and LL/2 tumor tissues derived from subcutaneous mouse models of renal cell carcinoma, colon carcinoma, and lung carcinoma, respectively. Additionally, we applied this panel to a 4T1 orthotopic primary mammary tumor and 4T1 lung metastasis formed via tail vein injection. The multiplex panel included antibodies detecting CD3 and CD8 as T cell markers, F4/80 as a myeloid cell marker, the immunosuppressive receptor PD-1, as well as its ligand PD-L1, pan-keratin as a tumor mask, and DAPI as a nuclear counterstain. We characterized the localization of tumor-infiltrating immune cells, as well as trends in the coexpression and frequency patterns of immunosuppressive proteins. This study strives to better understand the underlying differences in the immunologic landscapes of these tumors, which in turn has implications for researchers studying responses to immunotherapeutic approaches and combination strategies in murine models of cancer.

P.E1.02.14
REAlease® immunomagnetic separation technology with reversible labeling for positive selection of leukocytes

*P. Steinbrück, **C. Evaristo**, D. Vorholt, J. Pankratz, Z. Yu, M. Assenmacher, C. Dose;*
Miltenyi Biotec, Bergisch Gladbach, Germany.

Immunomagnetic enrichment of leukocytes is an important technique in both research and clinical applications. Current enrichment strategies using magnetic labeling of the target cells allow for highly efficient isolation, but in some instances removal of residual cell surface labeling after isolation is of high interest. To this end, we have combined the benefits of positive selection by MACS® Technology, the proven state-of-the-art method for the isolation of functional, viable cells, with a novel technology enabling the removal of both superparamagnetic beads and antibody fragments. REAlease® Technology provides an easy and fast solution for the highly specific isolation of unlabeled leukocytes directly from PBMCs. Separation based on this technology results in high purities of e.g. $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD56^+$ cells of around 95%. Here, we present our latest results on cell separation with REAlease Technology validating activation, proliferation and re-labeling of important cell subsets such as $CD4^+$ and $CD8^+$T cells, as well as $CD19^+$ B cells. Target cell isolation did not induce cell activation while upon stimulation the cells were properly activated indicating preserved cell physiology.

P.E2.01 How to handle big data?

P.E2.01.01
Biostatistics - Association Between Age, Occupation & Morbidity, using publicly available NIH longitudinal study data

***S. H. Cho**;*
-, -, United States.

According to the NIH longitudinal study data publicly available, there has been a significant association between Age, Occupation and Profession. It has been found that those who were working as Professionals & in Related Occupations, has been found to be recorded as dead in their late 20s, while occupations such as Service Occupations, as well as, people in Office & Administrative Support Occupations, seem to have seconded the high mortality rate. Farming, Fishing, and Forestry seem to have the least number of people in the public death records. It might mean that people in these occupations were never in the public death records or there had been least number of deaths in these occupations.
Acknowledgements - Harvard Medical School: Global & Continuing Education The National Longitudinal Mortality Study is a collaborative effort between the US Census Bureau and the National Heart, Lung, and Blood Institute (NHLBI), National Cancer Institute (NCI), National Institute on Aging (NIA), and the National Center for Health Statistics (NCHS). The views expressed in this paper are those of the authors and do not necessarily reflect the views of the Census Bureau, NHLBI, NCI, NIA or NCHS.

P.E2.01.02
On the feasibility of mining CD8+ T cell receptor patterns underlying immunogenic peptide recognition

***N. De Neuter**[1], W. Bittremieux[1], C. Beirnaert[1], B. Cuypers[1], A. Mrzic[1], P. Moris[1], A. Suls[2], V. Van Tendeloo[3], B. Ogunjimi[4], K. Laukens[1], P. Meysman[1];*
[1]Adrem Data Lab, Antwerpen, Belgium, [2]Center for Medical Genetics, Antwerpen, Belgium, [3]Laboratory of Experimental Hematology, Antwerpen, Belgium, [4]Centre for Health Economics Research & Modeling Infectious Diseases Lab, Antwerpen, Belgium.

Current T cell epitope prediction tools are a valuable resource in designing targeted immunogenicity experiments. They typically focus on, and are able to, accurately predict peptide binding and presentation by major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells. However, recognition of the peptide-MHC complex by a T cell receptor is often not included in these tools.
We developed a classification approach based on random forest classifiers to predict recognition of a peptide by a T cell and discover patterns that contribute to recognition. We considered two approaches to solve this problem: (1) distinguishing between two sets of T cell receptors that each bind to a known peptide and (2) retrieving T cell receptors that bind to a given peptide from a large pool of T cell receptors.
Evaluation of the models on two HIV-1, B*08-restricted epitopes reveals good performance and hints towards structural CDR3 features that can determine peptide immunogenicity. These results are of particular importance as they show that prediction of T cell epitope and T cell epitope recognition based on sequence data is a feasible approach. In addition, the validity of our models not only serves as a proof of concept for the prediction of immunogenic T cell epitopes but also paves the way for more general and high performing models.
This research was funded by the University of Antwerp [BOF Concerted Research Action] and the Research Foundation Flanders (FWO) [Personal PhD grants to NDN (1S29816N), PMo (1141217N), BC (11O1614N)]

P.E2.01.03
TCRex: a webtool for the prediction of TCR-epitope recognition

***S. Gielis**, N. De Neuter, W. Bittremieux, V. Van Tendeloo, B. Ogunjimi, K. Laukens, P. Meysman;*
University of Antwerp, Antwerpen, Belgium.

To date, multiple immunoinformatics tools have been created with the goal to achieve a better understanding of immunological processes. Although great tools exist for the prediction of epitopes and their binding to MHC molecules, we are still lacking useful tools for the prediction of epitope-MHC recognition by TCRs. Hence, we propose TCRex, a tool to investigate TCR recognition of epitopes.
This tool is based on our prior work related to the feasibility of predicting TCR-epitope recognition using TCRβ sequences. In this study, we showed that a random forest classifier trained to predict TCR-epitope interactions from TCR amino acid physicochemical properties can achieve a high accuracy. We are extending this work into a toolbox trained on a large dataset containing epitopes from different viruses, such as HIV-1 and EBV, and tumour cells. To this end, we collected epitope-specific human TCRβ sequence data containing information about the CDR3 sequences and the corresponding V-and J-genes. Random forest classifiers are trained on this data and kept if they report a sufficiently high performance in a cross-validation setting. These classifiers will be made freely available in a webtool, called TCRex.
TCRex will be useful to make predictions on newly gathered experimental TCRβ sequence data. As such, it will aid researchers in the development of more specific epitope-TCR binding assays and the elucidation of T cell repertoire targets.

P.E2.01.04
IUPHAR Guide to IMMUNOPHARMACOLOGY

***S. D. Harding**[1], E. Faccenda[1], A. J. Pawson[1], J. L. Sharman[1], C. Southan[1], S. P. Alexander[2], S. Anderton[1], C. Bryant[3], A. Davenport[3], C. Doerig[4], D. Fabbro[5], F. Levi-Schaffer[6], M. Spedding[7], J. A. Davies[1];*
[1]University of Edinburgh, Edinburgh, United Kingdom, [2]University of Nottingham, NOttingham, United Kingdom, [3]University of Cambridge, Cambridge, United Kingdom, [4]Monash University, Melbourne, Australia, [5]PIQUR Therapeutics, Basel, Switzerland, [6]Hebrew University of Jerusalem, Jerusalem, Israel, [7]Spedding Research Solutions, Le Vesinet, France.

Immunity, inflammation and infection have become priority areas in drug discovery research. Most chronic diseases, including ageing, have an immune-inflammatory component; auto-immunity is a serious problem, and the progress of infections depends on immune and inflammatory responses.
The International Union of Basic and Clinical Pharmacology (IUPHAR) have developed a new online resource called the IUPHAR Guide to IMMUNOPHARMACOLOGY (GtoImmuPdb; www.guidetoimmunopharmacology.org) that aims to improve data exchange between immunology and pharmacology expert communities, to better support research and development of drugs targeted at modulating immune, inflammatory or infectious components of disease.
Supported by the Wellcome Trust, GtoImmuPdb is an extension to the existing IUPHAR/BPS Guide to PHARMACOLOGY (GtoPdb; www.guidetopharmacology.org), a joint initiative between IUPHAR, the British Pharmacological Society (BPS) and the University of Edinburgh. It is an open-access and regularly updated resource, providing a unique access point that is user-friendly to immunologists and pharmacologists alike. Its integration with GtoPdb comes with enhanced search mechanisms and new ways to browse and visualize immunological data. Details of the expansion are provided in our recent update in the 2018 NAR Database Issue paper (1).
The data in the IUPHAR Guide to IMMUNOPHARMACOLOGY, and its parent database, are sourced from peer-reviewed primary literature and their content is curated by committees containing >500 global expert contributors.
1. Harding SD et al. (2018). The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. *Nucleic Acids Res*, 46(D1), D1091-D1106.

P.E2.01.05

Computational search of active compounds for SH2

V. Hurmach[1], *A. Varnek*[2], *M. Platonov*[3], *Y. Prylutskyy*[1];
[1]*Taras Shevchenko National University of Kyiv, Kyiv, Ukraine,* [2]*University of Strasbourg, Strasbourg, France,* [3]*Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine.*

Search for the new chemical structures with specific biological activity is a problem, which requires usage of the latest achievements in molecular modeling technologies. It is well known that SH2 domains are involved in intracellular signaling pathways. Their dysfunction is related with such cancer diseases as Basal cell carcinoma, T cell acute lymphoblastic leukemia and B cell lymphoma. The aim of this work - investigation of SH2 domain binding properties and searching for new potential active compounds in the whole SH2 domains class. All available structures of SH2 domain were divided to six groups with high/average level of conservation (group 1 - 91.25%, group 2 - 78%, group 3 - 47%, group 4 - 32%, group 5 - 39%, group 6 - 45%) and not significant Rmsd difference (0.8 Å, 1.16 Å, 1.6 Å, 1.9 Å, 2 Å, 1.4 Å).
Structure analysis showed that binding pocket contains 20-24 main amino acids: FLVRESETT (pTyr binding part), βB-sheet; KHYKIR (central part), βD-sheet; ITSR and ADGLC (hydrophobic pocket), βG-sheet and αB-helix. Seven most representative pockets were selected based on those sequences, and used for docking and pharmacophore study of the entire Enamine Ltd database (> 1 M compounds). The outcome of this stage is selection of 10463 compounds. To conclude, specific scaffolds have been identified as pTyr substitutes. In almost all cases the ligand tightly fills the phosphotyrosine binding site and creates hydrogen bonds with the key amino acids Arg and Lys.

P.E2.01.06

REAfinity recombinant antibodies can be used for Immunofluorescence staining of fixed mouse and human cells and tissues

A. Kinkhabwala, ***W. Muller****, T. D. Rockel;*
Miltenyi Biotec GmbH, Bergisch Gladback, Germany.

REAfinity™ recombinant antibodies are available from Miltenyi-Biotec conjugated to various fluorochromes. The main advantage of recombinant antibodies compared to hybridoma derived monoclonal antibodies is their reproducibility in their staining performance. One of the research areas in which monoclonal antibodies are utilised are multicolour immunofluorescence images from fixed cells or tissues. Here we show that REAfinity™ recombinant Antibodies can be used to stain various tissues and cells from mouse or human origin fixed by two fixation method and that they can be used in a multiplex manner. Examples of fixed tissue sections and single cell mixtures will be shown for a selected subset of REAfinity™ antibody staining demonstrating that REAfinity™ recombinant Antibodies can be used not only for flow cytometry but also for immunofluorescence staining of fixed cells and tissues.

P.E2.01.07

Robust prediction of peptide-MHC binding affinity with deep neural networks

V. I. Nazarov[1], *V. Tsvetkov*[2,1], *E. Ofitserov*[3];
[1]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russian Federation,* [2]*Pirogov Russian National Research Medical University, Moscow, Russian Federation,* [3]*Tula State University, Tula, Russian Federation.*

Current adaptive immunity research pose significant challenges to data analysis. One of the major challenges toward personalized vaccine design and other applications is the prediction of interaction between major histocompatibility complexes and their ligands. The emerging field of mass spectrometry immunopeptidomics complements existing binding affinity data, although common machine learning models are trained mostly on binding affinity data alone. Moreover, available data is often noisy and redundant. Therefore models inferenced from this data don't reach high accuracy due to data bias and corruption. In order to address both issues deep neural network was designed and trained on both binding affinity and mass spectrometry data. Specific architecture and learning process modifications were introduced to increase model robustness and to handle corrupt data. Altogether these advantages of designed deep learning algorithm allowed us to compete with state-of-the-art models, e.g. netMHCpan, and achieve comparable performance in at least 10 times less computation time.

P.E2.01.08

Identification of mutations associated with autoinflammatory diseases by Next Generation Sequencing

I. Olivas Martinez*, M. Montes Cano, L. Gonzalez Garcia, J. Bernabeu Wittel, L. Fernandez Silveiro, M. Camacho Lovillo;*
Hospital Virgen del Rocio, Sevilla, Spain.

The aim was to evaluate a patient with inflammatory symptoms and mild immune disorders using *Next Generation Sequencing* (NGS). Patient is a 27-year-old man with chilblain lesions that appear in winter and recurrent febrile episodes who was subjected to biochemical analysis, imaging techniques (Pulmonary CT scan) and autoimmunity studies (ANA, ANCA). A genetic study was also performed by NGS with a panel of 12 genes related to autoinflammatory diseases: *NLRP3*, *TNFRSF1A*, *MVK*, *PSTPIP1*, *MEFV*, *NOD2*, *CECR1*, *TMEM173*, *IL1RN*, *IL36RN*, *LPIN2* and *NLRP12*. Patient's DNA was amplified by multiplex PCR and sequenced using Ion Torrent platform. Mutations identified were confirmed by Sanger methodology. Imaging and autoimmunity studies were negative, but the presence of characteristic lesions, hepatic enzymes increased, neutrophilia and lymphopenia, made clinicians suspect an autoinflammatory SAVI-like syndrome (STING-Associated Vasculopathy with onset in Infancy). Four heterozygous mutations associated with autoinflammatory diseases were found: in exon 9 of *MEFV* gene with amino acid change I591T, in exon 11 of *MVK* gene with change V377I, in exon 7 of *LPIN2* gene with change A331S and in exon 5 of *TMEM173* gene, producing the change F153V. All these mutations were classified as non-pathogenic by the statistical analysis tool PolyPhen-2. F153V had not been described before, being located next to a pathogenic mutation (N154S), so we can't rule out its possible role in the above-mentioned pathology. Identification through NGS of 4 different mutations related to autoinflammatory syndromes in the same patient allows to extend the spectrum of mutations and phenotypes associated with these immune disorders.

P.E2.01.09

Novel miRNAs revealed in bovine macrophages stimulated with pathogen associated molecular patterns

F. N. Toka[1,2], *F. Smaltz*[1], *L. Szulc-Dabrowska*[2], *M. B. Mielcarska*[2], *P. Nielsen*[1], *M. Bossowska-Nowicka*[2];
[1]*Ross University School of Veterinary Medicine, Bassesterre, Saint Kitts and Nevis,* [2]*Faculty of Veterinary Medicine, SGGW, Warsaw, Poland.*

Introduction: MicroRNAs influence biological processes during development, cell differentiation and infection. We have mimicked bacterial and viral infection with pathogen associated molecular patterns to assess the early phase expression of miRNAs in a bovine macrophage cell line Bomac.
Material and Methods: Cells were stimulated with CpG or poly(I:C) for 6 h, and miRNAs isolated using an enrichment protocol and sequenced in Illumina system. Data were analyzed in miRDeep2. Read counts for mature miRNAs were input into R for data pre-processing and statistical analysis using Bioconductor.
Results: The 9 samples had 18-31 million reads. MiRNAs with 0.5 CPM in at least 3 samples were filtered out, leaving 1,318 miRNAs, comprised of 671 novel miRNAs, 564 known miRNAs that mapped to the genome and 83 known miRNAs that could not be mapped to the genome. Nine miRNAs were compared with the mRNAs; the overall FDR threshold was 0.35. Of these nine, seven were upregulated by CpG only and two were upregulated by poly(I:C) only. MiRNAs negatively regulate their targets, we looked for mRNAs with ≤ -0.75 Pearson correlation coefficients between mRNA expression and miRNA expression. 580 mRNAs were negatively correlated with one or more of the 7 CpG miRNAs and 507 mRNAs were negatively correlated with one or more of the 2 poly(I:C) miRNAs. Nine novel miRNAs were validated by Taqman small RNA assays and found to be expressed at levels that correlated with sequencing data.
Conclusion: We have discovered novel bovine miRNAs that may influence gene expression in infected bovine macrophages.

P.E2.01.10

The prediction of specific antibody- and cell-mediated responses using baseline immune status parameters of individuals received measles-mumps-rubella vaccine.

D. Grebennikov[1,2], ***A. Toptygina***[3,4], *G. Bocharov*[2];
[1]*Moscow Institute of Physics and Technology (State University), Moscow, Russian Federation,* [2]*Marchuk Institute of Numerical Mathematics of the Russian Academy of Sciences, Moscow, Russian Federation,* [3]*G.N.Gabrichevsky Research Institute for Epidemiology and Microbiology, Moscow, Russian Federation,* [4]*Lomonosov Moscow State University, Moscow, Russian Federation.*

The successful vaccination implies the induction of effective specific immune responses. We intend to find biomarkers among various immune cell subsets, cytokines and antibodies which could be used to predict the levels of specific antibody- and cell-mediated responses after measles-mumps-rubella vaccination. To do this, we measured 59 baseline immune status parameters (frequencies of 42 immune cell subsets, levels of 13 cytokines, immunoglobulins) before vaccination and 13 response variables (specific IgA and IgG, antigen-induced IFN-γ production, CD107a expression on CD8+ T-lymphocytes, and cellular proliferation levels by CFSE dilution) 6 weeks after vaccination for 19 individuals. Statistically significant Spearman correlations between some baseline parameters and response variables were found for each response variable ($p<0.05$). Due to the low number of observations relative to the number of baseline parameters and missing data for some observations, the automatic stepwise procedure of minimal adequate multivariable linear regression selection without overparameterization is unfeasible. To reduce the number of candidate parameters, we manually selected for each response variable 5 parameters among the parameters which correlated with response variable with $p<0.2$, and which were as much as possible uncorrelated between each other. Given the manually chosen candidate parameters, we managed to identify the minimal adequate predictive multivariable linear regression models of post-vaccination antibody- and cell-mediated responses with up to 5 predictors in each model. The work is supported by the Russian Foundation for Basic Research (grant 17-01-00636).

P.E2.01.11

Exploring large-scale flow cytometry data for identification of immune-related genes

I. Treise[1,2], *Y. Cho*[1], *G. Miller*[1], *H. Fuchs*[1], *V. Gailus-Durner*[1], *M. Hrabě de Angelis*[1], *D. H. Busch*[2];
[1]*Institute of Experimental Genetics, München/Neuherberg, Germany,* [2]*Institut für Medizinische Mikrobiologie, Immunologie und Hygiene, München, Germany.*

Immune-related diseases such as autoimmune or inflammatory disorders are a common clinical problem, but the underlying genetic factors are not yet fully understood. A large-scale approach is required to characterize protein-coding genes, their functional evidence and their relevance for immune system-related diseases. The International Mouse Phenotyping Consortium (IMPC) aims at generation and phenotypic characterization of knockout mouse lines of all coding genes. Within the Immunology Unit of the German Mouse Clinic, we systematically analyzed flow cytometry data of mice from the IMPC resource for abnormalities in the immune cell composition of the spleen. Multicolor flow cytometry is a powerful tool for simultaneous acquisition of a vast diversity of immune cell populations.
Components of both the innate and adaptive immunity were assessed: T, B, and NK lymphocytes and their respective subpopulations, as well as myeloid cell subsets, e.g. monocytes, granulocytes, macrophages and dendritic cells. Here, we show multiparametric analysis of large-scale flow cytometry data of more than 150 mutant mouse lines. We use statistical tools to visualize relations of the different immune cell subpopulations and to identify genes relevant for immune disorders.

P.E2.01.12

T- and B-cell receptor repertoire sequencing: quality control and clone identification

B. D. C. van Schaik[1], *P. L. Klarenbeek*[1], *M. E. Doorenspleet*[1], *S. Pollastro*[1], *A. Musters*[1], *G. Balzaretti*[1], *R. E. Esveldt*[1], *F. Baas*[2], *N. de Vries*[1], *A. H. van Kampen*[1,3];
[1]*Academic Medical Center, Amsterdam, Netherlands,* [2]*Leiden University Medical Center, Leiden, Netherlands,* [3]*University of Amsterdam, Amsterdam, Netherlands.*

Next-generation sequencing (NGS) of T- and B-cell receptor repertoires was introduced in 2009 as a new powerful tool in immunology research. Repertoire sequencing has found various applications such as the elucidation of plasmablast antibody response to influenza vaccination, the analysis of T-cell receptor diversity in systemic lupus erythematosus, and discovery of invariant T cells. Sequencing of repertoires has specialized experimental protocols and subsequent bioinformatics analysis of the data.
We have developed a data analysis strategy to analyse lymphocyte repertoires of (clinical) samples. To identify clones from this NGS data we use a set of sequence motifs, instead of the Cys104 and Phe/Trp118 to determine the CDR3 region and, subsequently, assign the V and J genes. After each sequence run and for every project we check for sample contamination by comparing the top 10, 25 and 100 most abundant clones between all samples.
In the past ten years we have performed ~90 NGS experiments with 100-200 samples per run. The data analysis is performed on a community cloud and the process is automated using a pilot job framework. Meta data is kept about the samples and is stored together with the data in a research data management system to enable reuse of these samples in future projects.
Grants: This work was carried out on the Dutch national e-infrastructure with the support of SURF Cooperative. P.L.K. was funded by a PhD scholarship of the AMC Graduate School.

P.E2.01.13

Arbitrary transformation of flow cytometry data for multivariate analysis may produce misleading immunological information

S. van Staveren[1,2], *G. H. Tinnevelt*[3,2], *R. Folcarelli*[3], *E. Cadot*[3], *J. J. Jansen*[3], *N. Vrisekoop*[1], *L. Koenderman*[1], *O. F. van den Brink*[2];
[1]*UMC Utrecht, Utrecht, Netherlands,* [2]*TA-COAST, Amsterdam, Netherlands,* [3]*Radboud University, Nijmegen, Netherlands.*

Introduction: The flow cytometry field has evolved rapidly, allowing the measurement of 30-50 parameters per cell. This has led to a tremendous increase in multivariate information. Manual gating is insufficient to extract all this information. Therefore, multivariate analysis (MVA) methods have been developed like cluster methods SPADE and FlowSOM and dimensionality reduction methods viSNE, FLOOD, DAMACY and ECLIPSE. To aid interpretation, the data are often transformed logarithmically before MVA.
Rationale: We studied the consequences of different transformations of flow data in datasets containing negative intensities caused by background subtractions and compensation, as logarithmic transformation of negative data is not possible. Alternative transformations such as biexponential, or logicle, and hyperbolic arcsine transformations allow linearity around zero, whereas higher (positive and negative) intensities are logarithmically transformed.
Results: To define the linear range, a parameter (or co-factor) must be chosen. The type of transformation and the concomitant chosen parameter have great impact on the MVA results. In some cases, peak-splitting is observed, producing two distributions in an actual homogeneous population around 0. This may be misinterpreted as the presence of multiple cell populations. We applied various statistical methods to optimally choose the parameter of the transformation used.
Conclusion: Arbitrary or unconsidered transformation can lead to wrong conclusions for cluster methods as well as dimensionality reduction methods. Importantly, it should be noted that some MVA methods only support one transformation option with a pre-set parameter in their algorithms. We recommend to transform flow cytometry data separately per channel, to prevent peak-splitting.

P.E2.01.14

Knowing what is what: Artificial Intelligence for cell classification in cytometry

J. Verhoeff[1], *J. J. Garcia-Vallejo*[1], *S. Abeln*[2];
[1]*Cancer Center Amsterdam, Amsterdam, Netherlands,* [2]*VU university Amsterdam, Amsterdam, Netherlands.*

The advent of high parametric cytometry systems (10+ color flow cytometry and mass cytometry) has greatly increased the amount of data gathered from single cell suspensions. Classical gating misses out on multi-parametric relations not visible on sequential biaxial plots, leading to the creation of computational tools, capable of clustering cells in an unsupervised manner. However the identified clusters would still need interpretation by a skilled immunologist to label them. This work details our strategy to develop a cell classifier, suited for mass cytometry data. We hypothesize a supervised approach is preferable over the laborious process of identifying unknown clusters. By identifying known subsets the process of cluster analysis can be expedited. This information is then implemented in metaclustering steps to reach biologically relevant clusters. We developed a neural network logistical regression model based on softmax regression. Softmax regression is a generalized logistic function. This model took as input a small set of classically gated and labeled cells (500 of each label) from 1 sample per batch. Initial results indicate a success rate of >95% in training, test and validation sets. The abundance of subsets does not affect identification rate. Our current work has shown to accurately classify cells within a single batch of mass cytometry data, with a high success rate. Future efforts will be focused on implementing different information sources, such as gene expression data in the classifier. This could eliminate the necessity of expert gating in mass cytometry analysis.

P.E2.01.16

BRepertoire: A user-friendly webserver for analysing antibody repertoire data

D. Dunn-Walters[1], *C. Margreitter*[2], *F. Fraternali*[2];
[1]*University of Surrey, Guildford, United Kingdom,* [2]*King's College London, London, United Kingdom.*

The major goal of BRepertoire is to facilitate easily accessible, fast and responsive data partition comparisons in the analysis of the antibody repertoire. A powerful data preparation pipeline and a set of analysis functionalities including PCA, V(D)J gene usage and statistical comparisons enables the investigation of repertoires in many different ways and ultimately the identification of distinguishing features between sub-groups of data. As most functions provide plots in addition to the text results, a quick screening of the data and visual inspection of the results is also possible. We believe BRepertoire to be an invaluable tool for experimental immunologists who study immune repertoires. A unique functionality is flexible analysis of physico-chemical properties of amino acid sequences, moving towards a better understanding of receptor-antigen interactions. Since flexibility has been a major design target, BRepertoire will prove useful in a variety of applications as it does not rely on pre-defined format conventions and tries to make as few assumptions on the data as possible. Plots and data generated may be downloaded as image files and data tables in different formats. The server is accessible under http://mabra.biomed.kcl.ac.uk/BRepertoire.

P.E2.01.17

Single cell multidimensional analysis reveals activation-induced generation and maintenance of regulatory T cells during homeostasis and in tumour microenvironments

A. Bradley[1], *T. Hashimoto*[1], ***M. Ono***[2];
[1]*University of Tsukuba, Tsukuba, Japan,* [2]*Department of Life Sciences, London, United Kingdom.*

Background: T cell receptor (TCR) signalling initiates downstream transcriptional mechanisms for T cell activation and differentiation. FoxP3-expressing regulatory T cells (Treg) require TCR signals for their suppressive function and maintenance in the periphery. It is, however, unclear how TCR signalling controls the transcriptional programme of Treg, and whether and how Treg are generated from activated T cells.
Results: Here we dissect the transcriptomes of various T cell subsets using multidimensional and single cell analysis methods. We show that Treg are as activated as memory-phenotype T cells (Tmem) and effector T cells (Teff) at the transcriptomic level, and identify the common activation-dependent gene modules for these T cell subsets. Importantly, the major feature of Treg is that Foxp3 represses Runx1-associated, Tmem-specific activation-dependent genes, while Foxp3 sustains or enhances the expression of the common activation genes. Furthermore, by analysing single cell RNA-seq data of tumour-infiltrating T cells from melanoma patients, we show that activated T cells in the tumour microenvironments dynamically differentiate into two lineages of cells: Treg and T follicular helper (Tfh)-like cells. We identify the bifurcation point of Treg and Tfh-like differentiation, which specifically contains *IL2* producing cells. Notably, *CTLA4* is induced in both the Treg and Tfh-like lineages upon activation, while *PDCD1* is induced in the Tfh-like lineage only. We identify marker genes to distinguish these new populations.

Conclusion: Our study establishes the new model that Treg are dynamically generated from activated T cells in tumour microenvironments and during homeostasis. Future studies should analyse the temporal dynamics of Treg differentiation and T cell activation in vivo.
Funding: BBSRC

P.E3E4.01 From single cells to population dynamics / Cell communication and signaling in the immune system

P.E3E4.01.01
Memory T-cell kinetics: do long-lived memory T-cells exist?

*M. Baliu-Piqué[1], J. Drylewicz[1], L. Ravesloot[2], S. Otto[1], L. Borkner[3], X. Zheng[3], L. Cicin-Sain[3], A. Koets[2], K. Tesselaar[1], **J. A. M. Borghans**[1];*
[1]Laboratory of Translational Immunology, University Medical Center Utrecht, Netherlands, [2]Central Veterinary Institute, Wageningen UR, Netherlands, [3]Helmholtz Center for Infection Research, Braunschweig, Germany.

Immunological memory, a hallmark of adaptive immunity, can last for decades, but it remains controversial whether long-lived T-cells contribute to this long-lasting memory. It has been shown that memory T-cells from blood and lymph nodes maintain themselves through proliferation, and not by longevity of the individual cells. Until now, however, most knowledge on memory T-cell dynamics is based on circulating T-cells and using phenotypic memory markers. It has been argued that *antigen-specific* memory T-cells may be longer-lived than the bulk of memory-phenotype T-cells, and that memory T-cells with long lifespans may be located in the bone marrow (BM), and hence go unnoticed in studies based on blood. We investigated the maintenance of memory T-cells against mouse cytomegalovirus (mCMV) using *in vivo* deuterium labelling. Since mCMV induces not only contracting but also inflating T-cell responses, which continue to expand over time, we could investigate whether *antigen-specific* memory responses, and in particular inflating responses, consist of long-lived memory T-cells. We found that the lifespans of antigen-experienced and memory-phenotype T-cells are comparable, and that even inflating T-cell responses are maintained by continuous cell renewal. We also investigated whether long-lived memory T-cells may be stored in the BM, by quantifying the *in vivo* dynamics of memory T-cells in BM, secondary lymphoid organs and blood of goats using deuterium labelling, and found that T-cells in BM are as short-lived as those in circulation. Taken together, our data support a dynamic model in which T-cell memory is maintained by homeostatic proliferation of relatively short-lived cells.

P.E3E4.01.02
Role of interleukin (IL)-12/15/18 and ruxolitinib in the phenotype, proliferation and polyfunctionality of human cytokine-preactivated NK cells.

*I. Terrén[1], I. Mikelez[1,2], I. Odriozola[1], A. Gredilla[1], J. González[1], A. Orrantia[1], O. Zenarruzabeitia[1], J. Vitallé[1], **F. Borrego**[1,3,4];*
[1]Biocruces Health Research Institute, Barakaldo, Spain, [2]CIC biomaGUNE, Donostia-San Sebastián, Spain, [3]Ikerbasque, basque Foundation for Science, Bilbao, Spain, [4]Basque Centre for Transfusion and HUman Tissues, Galdakao, Spain.

Interleukin (IL)-12, IL-15 and IL-18 preactivated NK cells, also known as cytokine-induced memory-like (CIML) NK cells, are a promising tool in cancer immunotherapy. We have studied the effect that combinations of IL-12, IL-15 and IL-18 have on the generation of human CIML NK cells. Our data points toward a major contribution of IL-15 to CIML NK cell mediated cytotoxicity, although the synergistic effect of the three cytokines grant them the best polyfunctional profile, that is, cells that simultaneously produce multiple cytokines such as interferon (IFN)γ, tumor necrosis factor (TNF)α and C-C motif chemokine ligand (CCL)3 and degranulate (CD107a). We have also analyzed the involvement of each cytokine and their combinations in the expression of homing receptors CXCR4 and CD62L, as well as the expression of CD25 and IL-2-induced proliferation. Furthermore, we have checked the effects of the Jak1/2 inhibitor ruxolitinib in the generation of CIML NK cells. We found that ruxolitinib-treated CIML NK cells express lower levels of CD25 than non-treated CIML NK cells, but show similar IL-2-induced proliferation. Moreover, we have also found that ruxolitinib-treated NK cells show reduced effector functions after the preactivation, and that these functions can be recovered after an expansion period of 4 days in the presence of low doses of IL-2. Altogether, our results describe the impact that each cytokine and the Jak1/2 pathway have in the phenotype, IL-2 induced proliferation and effector functions of human CIML NK cells. **Funding:** AECC-Spanish Association Against Cancer (PROYE16074BORR) and BIOEF-Basque Foundation for Research and Innovation-EiTB Maratoia (BIO14/TP/003).

P.E3E4.01.03
Characterizing the human Yellow Fever Vaccine immune response by gene expression profiling at single-cell resolution

***J. J. A. Calis**[1,2], S. A. Uhl[2], R. Caron[2], B. R. Rosenberg[2];*
[1]The Rockefeller University, New York, United States, [2]Icahn School of Medicine at Mount Sinai, New York, United States.

Introduction. An effective immune response relies on the coordinated activity of many cell types and associated functions. Transcriptomics methods enable detailed characterizations of these complex and dynamic processes. RNA-Seq and microarray studies of peripheral blood samples from human volunteers have revealed numerous gene expression networks and corresponding cellular functions that contribute to effective immunity. Recently developed high-throughput single cell RNA-Seq (scRNA-Seq) technologies offer great potential for further characterization of immune function at increased resolution. In measuring gene expression in individual cells, these methods can resolve different cell types in complex mixtures, to analyze gene expression of many cell types without *a priori* selection, and assess cell-to-cell heterogeneity.
Methods. We applied high-throughput scRNA-seq to characterize the human immune response to Yellow Fever Vaccine (YFV). YFV was administered to healthy volunteers and peripheral blood mononuclear cells (PBMC) were analyzed at 0, 3, 7, 14 and 42 days post-vaccination by scRNA-seq. We analyzed approximately 75,000 total immune cells using unsupervised clustering methods to identify different cell types and assess vaccine-elicited changes in gene expression.
Results. Preliminary results include a robust Interferon response at days 3 and 7 post-vaccination, with distinct interferon-stimulated gene (ISG) expression programs induced in different cell types. At later time points, we observed activated T and B cell expansions characteristic of the adaptive immune response to vaccination. Additional analyses have revealed numerous cell type-specific gene expression programs in the response to this highly effective and clinically important vaccine.

P.E3E4.01.04
Dietary lipids can modify the immune-related transcriptome in Atlantic salmon: a model organism

***M. Jalili**[1], Y. Jing[1], Y. Olsen[1], A. Bones[1], M. Ostensen[1], F. Buonocore[2], M. Gerdel[3], A. Pallavicini[3], G. Scapigliati[2];*
[1]NTNU, Trondheim, Norway, [2]University of Tuscia, Viterbo, Italy, [3]University of Trieste, Trieste, Italy.

Background Aquaculture nutrition has an important determining role in fish and human health. The type and amount of fatty acids may affect the immune response and there is a knowledge gap in this area. Our objective was to investigate the immunomodulatory effects of fish oil (FO), vegetable oil (VO) and phospholipid (PL) rich feedings in farmed Atlantic salmon juveniles. Methods and Materials After 12 weeks at aquaculture facility, pyloric caeca (PC) and liver (LV) tissues were dissected from fishes and RNA was extracted, cDNA libraries were prepared and sequenced according to standard protocols. The raw data were processed, mapped, quality-checked and analyzed statistically to compare different sampling time points (Day0, Day48 and Day94) and feedings (FO, VO and PL) in two type of tissues (PC, LV) in order to determine the significantly up- and down-regulated immune related genes and relative pathways. Results and discussion With regard to immunological related transcripts, there were limited differences among the diets and the immune modulation was very similar comparing the three different dietary interventions. Taking into account the sampling at Day48 and Day0, the highest up-regulated genes were related to complement system and immunoglobulins chains. The moderate modulated genes were of pathogen recognition receptors and triggering receptor expressed in myeloid cells (TREM-1). Although transcriptome of all markers and receptors was not changed, there was a remarkable evidence for modulation of gene networks and pathways related to innate (TLRs, NLRs, complement C1q and C3) and adaptive immunity (immunoglobulins, T cell receptors, phagocytes and opsonization factors).

P.E3E4.01.05
Defining the memory T cell cytotoxic mode of action with a high-resolution immune cell cytotoxicity assay on single cell level

***A. Knörck**, K. S. Friedmann, C. Backes, C. Kummerow, D. Alansary, G. Schwär, S. Renno, S. Zoephel, M. Hoth, E. C. Schwarz;*
Biophysics, Homburg, Germany.

Introduction: Following antigen recognition cytotoxic T lymphocytes (CTLs) undergo differentiation into various subtypes of effector and memory T cells. Effector Memory (T_{EM}) and Central Memory (T_{CM}) T cell subsets induce target cell death by at least two different lytic mechanisms. Death receptor mediated- or perforin/granzymeB mediated mechanisms induce target cell apoptosis and/or necrosis. Apoptotic and necrotic target cell death can be distinguished by a pCasper 3GR single immune cell cytotoxicity assay, which was used to identify the preferred mechanisms of target cell lysis of T_{EM} and T_{CM}.
Methods: Human $CD8^+$ T cells were isolated out of a SEA-stimulated PBMC population and sorted into $CD45RO^+/CD62L^-$ TEM, $CD45RO^+/CD62L^-$ TCM subpopulations. The cytotoxic repertoire and signaling of CTL subpopulations were characterized by qRT-PCR, intracellular perforin staining, calcium imaging and a multiplex bead array assay. The cytotoxic effector functions of these CTL subpopulations were further analyzed by a population based real-time killing assay and high-resolution cytotoxicity assay on single cell level.
Results: After in vitro SEA-stimulation of CTLs 15% of the $CD8^+$ population show a T_{EM} and 50% a T_{CM} phenotype. Although T_{EM} display a highly increased cytotoxicity and elevated expression of Perforin and other effector molecules, both subpopulations mainly rely on a perforin/granzymeB mediated killing mechanisms. Perforin mediated primary necrosis and slower apoptotic events were of less importance for both subpopulations.
Conclusion: Using a high-resolution immune cell cytotoxicity assay on single cell level sheds light on the kinetics, efficiency, and mode of T_{EM} or T_{CM} induced target cell lysis in great detail.

P.E3E4.01.06

Comparative analysis of transcriptomic data obtained from SCLC cells upon IFN-gamma response display great heterogeneity

A. M. KURSUNEL[1], *E. Z. TASKIRAN*[2], *G. ESENDAGLI*[1];
[1]*Department of Basic Oncology, Hacettepe University Cancer Institute, Ankara, Turkey,* [2]*Department of Medical Genetics, Hacettepe University Faculty of Medicine, Ankara, Turkey.*

Introduction: Small cell lung cancer (SCLC) which comprises 13% of all lung cancer cases is associated with aggressive tumor growth together with a tendency to early dissemination and distant metastasis. IFN-γ is the uppermost cytokine implicated in anti-tumor immunity. Although it plays a central role in the recognition and elimination of transformed cells, many immune regulatory pathways can also be induced. The aim of this project is to determine whether an IFN-γ-related immune activation occurs against SCLC cells and how SCLC cells cope with anti-tumor effects of IFN-γ. **Materials&Methods:** SCLC cell lines were co-cultured with PBMCs. T-cell proliferation, IFN-γ and IL-2 secretion were determined. Additionally, SCLC cell lines were exposed to recombinant IFN-γ and total RNA was extracted. RNA-seq library preparation and next-generation sequencing (NGS) were performed. NGS data were analyzed using various approaches (STRING, KEGG). **Results:** SCLC cells promoted the proliferation of T-cells within the PBMCs. IL-2 and IFN-γ levels were also elevated in supernatants collected from the SCLC:PBMC co-cultures. Differential effects of IFN-γ on each SCLC cell line was observed. Especially major immune regulatory pathways (such as PD-L1 and IDO1) were heterogeneously modulated. **Discussion:** These data indicates an adaptive resistance mechanism for SCLC since these cells initially allow T-cell activation and IFN-γ secretion. SCLC cells are responsive to IFN-γ while different immune regulatory pathways were induced. Thus, exposure to IFN-γ revealed alternative mechanisms employed by SCLC cells to cope with immune responses. These data might provide new insights for cancer biology and immune intervention therapies.

P.E3E4.01.07

Characterising the functional and genomic profile of CAR CD19 T cells using single cell analyses

F. Luciani[1], *L. Clancy*[2], *C. Cai*[1], *H. McGuire*[3], *E. Keoshkerian*[4], *D. Koppstein*[4], *E. Blyth*[2], *D. Gottlieb*[2], *K. Micklethwaite*[2];
[1]*School of Medical Sciences, Sydney, Australia,* [2]*Westmead Institute for Medical Research, University of Sydney, Sydney, Australia,* [3]*University of Sydney, Australia, Sydney, Australia,* [4]*Kirby Institute, UNSW Sydney, Sydney, Australia.*

Despite the great success of CAR T-cells in the treatment of blood malignancies the mechanisms underpinning long-term persistence and limited toxicity remain unknown. The presence of less-differentiated T-cells such as T-stem-cell-memory (T_{SCM}) have been correlated to long-term persistence and limited toxicity. A single cell (sc)RNAseq approach has been developed to identify and track post-infusion the transcriptional signatures and endogenous TCR of CAR-T cell subsets and to link these profiles to clinical data. Three CAR-T products have been administered with complete molecular remission in two patients with acute lymphoblastic leukaemia and complete metabolic remission in a third with diffuse large B-cell lymphoma. CAR-T cells generated using thePiggyBac transposon-system with IL-15 and co-cultured with peripheral-blood mononuclear cells showed between 5-10% of T_{SCM}cells. CAR-T cells expanded in blood, produced cytokine-mediated toxicities commensurate to tumour-burden, and had up to 200 CAR-T/µl blood at 3-months follow-up. scRNAseq from the total $CD3^+$or $CD8^+T_{SCM}$($CD45RA^+CD95^+CXCR3^+$) compartments had a median of 858-950 genes per cell, with multiple gene-clusters identified. T_{SCM}showed 4 clusters, one with gene profile consistent with long-term memory and enhanced respiratory capacity and fatty-acid oxidation, in line with recent findings on 4-1BB CAR-T compared to CD28CAR-T cells. Full-length TCR was identified in 20% of the cells, and at least one chain in 75% of the cells. Ongoing research on post-infusion blood samples seeks identification of T_{SCM}clones that are maintained in the blood. This study reveals heterogeneous population of cells in the CAR T product, which can confer long-term survival of cells in the patient and minimise toxicity

P.E3E4.01.08

TCR sequence motif based classification of CD4-CD8 cells

E. Ofitserov[1], *V. Tsvetkov*[2,3], *V. I. Nazarov*[3];
[1]*Tula State University, Tula, Russian Federation,* [2]*Pirogov Russian National Research Medical University, Moscow, Russian Federation,* [3]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russian Federation.*

The field of immunology has witnessed an exponential growth of data. For instance, accumulation of TCR sequencing data allowed classification of CMV status in patients. This is the very point where big data algorithms should step in. Nevertheless, conventional deep neural networks (NN) lack of interpretability due to poor algorithmic transparency. In order to combine interpretability and performance we sought to develop a comprehensive NN training algorithm for motif-based sequence classification. It incorporates differential sequence alignment that provides end-to-end learning without preprocessing. Short substrings with gaps - TCR motifs - are generated during model training that constitute the features for the classification. In other words generated sequences are the cell type associated motifs as well as a part of NN classification model. The method was applied to human TCR sequencing data to classify CD4 and CD8 cell subsets. This analysis revealed the CD4 and CD8 associated motifs with the mean accuracy of prediction at least 70% across all repertoires. Thus it is an intelligible way to discern the measurable difference between cell subsets.

P.E3E4.01.09

HLA Frequencies in a a population of Barranquilla, Colombia

C. H. Parga Lozano[1,2], *F. Torres*[1], *A. García*[1];
[1]*Universidad Libre, Barranquilla, Colombia,* [2]*Universidad del Atlántico, Barranquilla, Colombia.*

Introduction: Barranquilla is a city in northern Colombia. The city had high immigration in the last 200 years, mainly from Central Asia, Africa and Europe. That caused a significant miscegenation of the population along with native Amerindians in the region. Materials and methods: 43 individuals from Barranquilla were typed for the detection of the HLA-DRB1 and DQB1 alleles. The HLA allele frequencies of 738 chromosomes were analyzed and compared these with 9 Colombian Amerindian populations using genetic distances, neighborjoining dendrograms (NJ) and correspondence analysis. Results: The most frequent alleles were DRB1 * 15: 01(0,088889), DRB1 * 01: 01(0,077778), DRB1 * 04: 01(0,077778) and DQB1 * 06: 01(0,16667), DQB1 * 03: 02(0,15556), DQB1 * 05: 01(0,13333). Several new haplotypes were obtained. Of which the most frequent were DRB1 * 01: 01-DQB1 * 05: 01 and DRB1 * 04: 01-DQB1 * 03: 02. According to the NJ results and correspondence analysis, the population is not related to the Amerindians, due to its presence as a group in the NJ phylogenetic tree. Discussion: Based on these analyzes it was found that there is a close relationship between the indigenous people from Colombia, mainly with the indigenous from Sierra Nevada de Santa Marta. Several DRB1/DQB1 haplotypes were not reported previously and this population from Barranquilla, moreover this population is not related to any of the Amerindians directly, which suggests a miscegenation and therefore in future studies should be included mestizo, Caucasian, Asian and Black populations to obtain a better phylogenetic relationship.

P.E3E4.01.10

A novel statistical approach to monitor clonal antigen-specific T-cell responses using targeted RNA-seq

S. Pollastro[1], *M. De Bourayne*[2], *B. van Schaik*[1], *A. van Kampen*[1], *A. van Kampen*[1], *B. Maillère*[2], *N. de Vries*[1];
[1]*AMC, Amsterdam, Netherlands,* [2]*SIMOPRO, CEA, Saclay, France.*

Introduction: *In vitro* T cell stimulation assays are basic immunological tools used to investigate antigen-specific T cell responses. Here we explored different experimental set-ups to take this tool to the single clone level using next generation sequencing (NGS) based technologies and novel statistical approaches. **Methods:** We analyzed sequential samples - taken in triplicate - during 10 day peptide-based *in vitro* T cell stimulation assays using NGS-based T-cell receptor (TCR) repertoire analysis (NGS-TCR-RA) to identify, fingerprint and monitor antigen-specific T cell responses at the clonal level. **Results:** Reproducible gradual expansion of initially low-frequency T-cell clones was observed during the cultures. We developed a statistical approach to identify differentially expanded, candidate antigen-specific TCR clones by comparing the pre-stimulation and day 10 post-stimulation repertoire. We confirmed antigen-specificity of most significantly expanding clones using NGS-TCR-RA of T-cells sorted on CD40L expression upon short antigen re-challenge. **Conclusion:** NGS-TCR-RA is a robust, sensitive and reproducible tool for identification and monitoring of antigen-specific T-cell responses, performing statistical evaluation at the single clone level. Furthermore, based on clonal fingerprinting it is able to link *in vitro* analysis of clones to *in vivo* observations on T-cell responses. As such it constitutes a novel, powerful “omics” tool to fingerprint relevant B- and T-cells in adaptive immune responses.

P.E3E4.01.11

Communicome analyses identify the TNF/TRAIL receptor family as a potential determinant of virus control and CD4 T cells counts in natural chronic HIV infection.

M. Ruiz-Riol[1], *B. Oriol-Tordera*[1], *A. Llano*[1], *B. Mothe*[1,2], *P. Susana*[1], *C. Galvez*[1], *D. Berdnik*[3], *J. Martinez-Picado*[1,2,4], *C. Ganoza*[5], *J. Sánchez*[5], *G. Gómez*[6], *B. Clotet*[1,2], *T. Wyss-Coray*[3], *C. Brander*[1,4,7];
[1]*IrsiCaixa, AIDS Research Institute, Badalona, Spain,* [2]*Universitat de Vic - Universitat Central de Catalunya (UVic-UCC), Vic, Spain,* [3]*Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, United States,* [4]*Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain,* [5]*IMPACTA, Lima, Peru,* [6]*Universitat Politècnica de Catalunya-Barcelona Tech, Barcelona, Spain,* [7]*Aelix Therapeutics, Barcelona, Spain.*

The progressive loss of CD4 T cells in untreated HIV infection is incompletely understood. Apart from direct cytolytic effects of viral replication, “bystander” apoptosis triggered by soluble factors or membrane-bound host immune factors also contribute to cell death of infected and uninfected cells. To identify soluble plasma factors driving cell-death during chronic HIV infection, “communicome” profiles were assessed in HIV-infected, treatment-naïve individuals with high (>50,000, n=47) or low (<10,000 HIV RNA copies/ml, n=49) viral loads. Multivariate classificatory and regression model analyses for CD4 count prediction revealed that plasma levels of soluble death factors, most of them involved in TRAIL (tumor necrosis factor (TNF)-related apoptosis-inducing ligand) signaling, are critical for control of virus replication and CD4 loss.

In particular, plasma concentrations of two TRAIL decoy receptors (TRAILR3 and TRAILR4) were significantly correlated with peripheral blood CD4 T cell counts as well as viral load and proviral HIV-DNA copies (all p<0.01).
Total PBMC-based gene expression levels supported these associations further. An additional TNF ligand (TL1A; TNFSF15) and TNF receptor (DR3; TNFRSF25), were strongly related with CD4 counts. These results were validated in unrelated cohorts of seronegatives, recently HIV infected subjects (3-6 months post-seroconversion), longitudinally followed individuals tested 1 year before and after cART initiation and HIV controllers. The data identify several soluble plasma markers of the TNFR family that are related to HIV disease progression and which are involved in "bystander" cell death, thereby opening potential new targets for immune-based therapeutic interventions in HIV infection.

P.E3E4.01.12
A mathematical model of TCR signaling and its information transmission in a pool of low affinity ligands

***K. Saeki**, R. J. de Boer;*
Utrecht University, Utrecht, Netherlands.

T cells scan the surface of antigen presenting cells to detect foreign peptides on major histocompatibility complex (MHC). Recognition takes place via the binding of T cell receptor (TCR) to peptide-MHC and the transduction of downstream signaling cascades. To induce a proper immune response, it is essential that T cells are activated when they bind to foreign peptides and not when confronted with self-peptides. Although T cells that have high affinity to self-peptides are deleted during development in the thymus, T cells in the periphery should still discriminate foreign peptides with high affinity to its TCR from the many and abundant low affinity self peptides. Failures in this discrimination result in either infection or autoimmune diseases.Many models have been suggested to explain this, however, it is not clear that how peptide discrimination by TCRs is disturbed in the background of many low affinity self-peptides that could antagonize signaling. Here we simulate the TCR signaling model developed by Francois et al. 2013 *PNAS* with low affinity ligands, and quantify the ability of ligand discrimination using mutual information. The mutual information indicates the maximum number of input signal values that a signaling pathway can reliably resolve. The results show that ligand discrimination works only in the presence of antagonists with very low affinity, which suggests that negative selection of immature T cells should be very strict. We will also discuss how the selection of immature T cells with different strength of TCR signaling results in different T cell differentiation.

P.E3E4.01.13
Development of recombinant antibodies: highly reproducible with tailored specificity

***M. Sassi**, A. Wittmann, A. Symonds, R. Adams, A. Solache, B. Hamilton;*
Abcam plc, Cambridge, United Kingdom.

Recombinant Antibodies are fundamental tools in both basic and clinical research of Immunological targets. However, an increasing number of studies have shown that not all antibodies are specific, which leads to a lack of experimental reproducibility.
To provide antibodies that have excellent specificity and reproducibility, we have engineered recombinant versions of our RabMAb® rabbit monoclonal antibodies. Recombinant antibodies are manufactured by cloning the immune-specific heavy and light antibody chains into a high-yield mammalian expression vector or they can be produced from an existing hybridoma.
The technology combines the superior antigen recognition of a rabbit immune system with the specificity and consistency of a monoclonal antibody. Our RabMAb® products are validated in key applications (western blot, IHC, ICC/IF, IP, and flow cytometry) and include several relevant targets in immunology and immuno-oncology research. Successfully validated products will be produced in numerous formats (Fluorescent conjugates, PBS formulations) to allow their use in many types of in vivo and in vitro studies.
Our phage display technology is an alternative method to generate high-affinity binders against difficult proteins, small molecules, and toxins. This *in vitro* approach is based upon a large library of bacteriophage particles, each carrying the genetic information and the unique phenotypic binding function of one antibody clone.
Utilizing both *in vivo* and *in vitro* technologies, we can deliver high quality antibodies to all targets. The advantages of recombinant antibodies over both traditional monoclonal and polyclonal antibodies are increased in consistency and reproducibility, high affinity and specificity, ease of scalability.

P.E3E4.01.14
Quantifying immune cell subsets in living cultures over time using IncuCyte® live-cell analysis

***C. Szybut**[1], N. Bevan[1], H. Campwala[1], L. Kelsey[1], N. Dana[2], T. Jackson[2], N. Holtz[2], E. Endsley[2], T. Dale[1], D. Trezise[1];*
[1]Essen BioScience, a Sartorius Company, Welwyn Garden City, United Kingdom, [2]Essen BioScience, a Sartorius Company, Ann Arbor, United States.

CD surface markers have long been used to identify immune cell subsets and associate cells with certain immune functions. Typically, flow cytometry and specific fluorescently-labelled anti-CD antibodies are used for these analyses. Whilst extremely powerful, this approach does not readily afford insight into temporal changes or spatial interactions between cell populations in heterogeneous systems. Here we describe a novel labelling and analysis strategy to enable long-term, non-invasive quantification of immune cells based on IncuCyte® live-cell imaging.
An Fc-targeted anti-mouse Fab fragment conjugated to a green-emitting fluoroprobe (IncuCyte FabFluor-488) was used to tag antibodies to cell surface markers. Addition of the FabFluor488-antibody complex to living cells, including OptiGreen background suppressor, produced fluorescent labelling that was sufficiently bright and stable to allow repeated measurements for >4 days without perturbing cell morphology or growth. With new image analysis and visualisation tools, individual cells were segmented from the phase-contrast image and quantified cell by cell for fluorescence. The method was validated by comparing the phase cell counts over time with nuclear fluorescence values in proliferating Jurkat-Nuclight RFP cells. In PBMCs, the anticipated frequencies of lymphocyte subpopulations (CD4, CD8, CD45) were detected using this method. Cell subsets can be gated for further analysis determining dynamic changes in response to stimuli, e.g. increase in CD71+ cells following anti-CD3/IL-2 activation.
FabFluor antibody labelling and live-cell imaging enables long-term tracking and analysis of immune cell subsets in living cultures over time. This method should prove powerful in analyses on dynamic heterogeneous cell models and for studying cell-cell interactions.

P.E3E4.01.15
Define the dynamic protein landscape during early of human Th17 cell differentiation

***S. K. Tripathi**, T. Välikangas, M. Khan, A. Shetty, S. D. Bhosale, R. Moulder, E. Komsi, V. Salo, R. De Albuquerque, O. Rasool, L. E. Elo, R. Lahesmaa;*
Turku Centre for Biotechnology, Turku, Finland.

Th17 cells play key role in the pathogenesis of inflammatory and autoimmune diseases and in various cancer. Hence, it is critical to unveil molecular signatures driving the differentiation of Th17 cells in order to understand their regulation during diseased state. We performed label-free mass spectrometry based quantitative proteomics analyses to reveal the Th17 cell-specific proteomic signature regulating Th17 cell differentiation and function in human. We compared the human Th17 proteomics data generated in this study with our previously published data on the transcriptomic profiles during human Th17 differentiation that revealed the degree of similarities and differences between the transcript and the protein levels. Furthermore, we validated a panel of selected proteins with known and unknown functions in Th17 cell differentiation. To our knowledge, this study is the first to map the global protein landscape during early human Th17 cell differentiation.

P.E3E4.01.16
Inflamed tissue contributes to the emergence of auto-reactivity in granulomatosis with polyangiitis

***G. Weppner**[1], O. Ohlei[2], C. Hammers[3], K. Holl-Ulrich[4], K. Hasselbacher[5], G. Riemekasten[1], S. Ibrahim[3], A. Recke[3], P. Lamprecht[1], A. Müller[1];*
[1]Department of Rheumatology & Clinical Immunology, Lübeck, Germany, [2]LIGA, Lübeck, Germany, [3]Department of Dermatology, Lübeck, Germany, [4]Institute of Pathology, Marienkrankenhaus, Hamburg, Germany, [5]Department of of Otorhinolaryngology, Lübeck, Germany.

Circulating anti-neutrophilic cytoplasmic autoantibodies targeting proteinase 3 (PR3-ANCA) are a diagnostic and pathogenic hallmark of granulomatosis with polyangiitis (GPA). It is, however, incompletely understood if inflamed tissue supports presence and emergence of PR3-ANCA$^+$ B-cells. In search of such cells in inflamed tissue of GPA, immunofluorescence staining for IgG and a common PR3-ANCA idiotype (5/7 Id) was undertaken. To gain insight into surrogate markers possibly indicative of a PR3-driven antibody response at inflamed sites, a meta-analysis comprising IGVH and IGVL genes derived from respiratory tract tissue of GPA (231 clones) was performed.
Next generation sequencing-based IGHV genes derived from peripheral blood of healthy donors (244.353 clones) and previously published IGLV genes (148 clones) served as controls. For comparison, Ig genes of murine and known human monoclonal anti-PR3 antibodies were analyzed. Few 5/7 Id$^+$ / IgG$^+$ B-cells were detected in inflamed tissue of GPA. IGHV and IGLV genes derived from inflamed tissue of GPA displayed altered V(D)J usage, contributing to prolonged complementarity determining region 3 (CDR3) in the IGHV genes. Further, selection against amino acid exchanges was prominent in the framework region of IGHV genes derived from inflamed tissue of GPA. Comparing V(D)J rearrangements and deduced amino acid sequences of the CDR3 between anti-PR3 antibodies and Ig clones derived from inflamed tissue of GPA, yielded no identities and few similarities. Thus, few PR3-ANCA$^+$ B-cells were found in inflamed tissue of GPA. Notably, the search for clones producing PR3-ANCA IgG in inflamed tissue might require methods that can detect rare clones.

P.E3E4.01.18

Extracellular Vesicles Derived From Mesenchymal Stromal Cells Modulate Immune Cells generating a Regulatory Profile

F. Franco da Cunha[1], *V. Andrade-Oliveira*[2], *D. Candido de Almeida*[1], *T. Borges da Silva*[1], *M. Cenedeze*[1], *M. Ioshie Hiyane*[2], *R. Aparecida Cavinato*[2], *N. Olsen Saraiva Camara*[2];
[1]*Federal University of São Paulo- Unifesp, SAO PAULO, Brazil,* [2]*University of Sao Paulo, SAO PAULO, Brazil.*

Mesenchymal Stromal Cells (MSC) can generate immune tolerance due to their inhibitory activity toward T and B cells, dendritic cells (DC) and natural killer cells. Extracellular vesicles (EVs) are one of the main mechanisms by which MSC exert their actions. In this study, we hypothesize that EVs from MSC can, by themselves, modulate the immune response, generating a regulatory profile.EVs were obtained by ultracentifugation. DCs from bone marrow were expanded with GM-CSF. The incorporation by DCs and Tcells was detected by confocal captures. EVs-treated DC were used as stimulus for Tcell differentiation assay. Tcell proliferation assay was performed with anti-CD3 and anti-CD28 antibodies. Tcell polarization was evaluated in naïve TCD4 proliferation with specific cytokines. The expression of surface markers was detected by flow cytometry (FACS) and the cytokines were detected by RT-PCR, ELISA, FACS and confocal microscopy. A miRNA PCR array was performed to evaluate miRNA profile. EVs were incorporated by DCs and lymphocytes. EVs treatment didn't affect the expression of surface markers on DCs, but altered their function. TCD4 cells in the presence of EVs-treated DCs demonstrated lower differentiation toward Th1 cells. EVs led to lower Tcell proliferation and lower Th1 differentiation. Interestingly, the addition of EVs in a Th1 polarization increased the expression of Foxp3, generating some $IFNg^{+}FoxP3^{+}$ cells. The treatment of EVs with Rnase abolished some effects, suggesting a role of RNAs/miRNAs in this regulation. Some miRNAs were regulated after EVs treatment. Our data suggests that MSC-EVs are able to manipulate immune cells, generating a regulatory profile, specially in Tcells. Support by CNPq, FAPESP.

P.E3E4.01.19

Myeloid cells mediates the accelerated growth of tumor in high-fat diet-fed mice

T. Tran, M. Montabord, B. Esposito, E. Tartour, ***S. Potteaux****;*
INSERM U970, Paris, France.

Cancer and cardiovascular diseases (CVDs) are the leading causes of mortality worldwide. Components of the metabolic syndrome have been shown to aggravate both disease conditions. Here, we investigated the role of early dyslipidemia on melanoma development in mice with or without pre-atherosclerosis conditions. C57Bl6J mice were fed with a pro-atherogenic high fat diet (HFD: 15% FAT and 1.25% cholesterol) or a control chow diet starting two weeks before subcutaneous injection of B16-F10 melanoma cells. Our data showed that mice on a HFD displayed accelerated melanoma progression independently of cholesterol levels and insulino-resistance. Kinetic analysis revealed an increase of systemic inflammation and accumulation of myeloid cells in the growing tumor, in mice on HFD. Transcriptome analysis showed a switch in immune responses in the tumor microenvironment. Leukocytes isolated from the tumor of HFD-treated mice had lost their capacity to inhibit tumor cell proliferation as compared with chow diet-fed mice. Selective depletion of monocytes/macrophages with clodronate liposomes, or myeloid-derived suppressor cells with an anti-Gr1 antibody partially protected against melanoma progression under HFD. Mechanistic analysis demonstrated the implication of VEGF and IL-1beta in the protumoral effect of myeloid cells under HFD. Current experiments are now being performed to identify the implication of specific fatty acid in the immuno-metabolic switches orchestrated in myeloid cells infiltrating the tumor at early time points. In conclusion, this transversal study should help understand in more details the communication between free fatty acids, immune cell activation and tumor development. It should as well allow proposing new therapeutic strategies to limit tumor growth in patients with metabolic syndrome.

P.E3E4.01.20

Deep phenotyping approach to reveal the novel platelet interaction with immunological cells

S. Kalyan*;*
ARUP Laboratories, Salt Lake City, United States.

In this study we have used the mass cytometry (Helios CyTOF) technique combined by the dimensionality reduction analytical approach (utilizing R/Bioconductor) to reveal novel interaction between platelets and several immunological cells in normal healthy donors whole blood. Using the power of CyTOF we first identified expression of novel surface marker on platelets. These expression were compared with the platelet expression data base and RNA-Seq data. We later explored the already known physical interaction between platelets and immunological cells such as monocytes, dendritic cells and NK cells, albeit at the subtype level. Platelets prefer classical monocytes and myeloid dendritic cells over non-classical sub-types for physical interaction. We also identified physical interaction between platelets and T and B lymphocytes. The study revealed that platelets prefer naive T and B cells over active and memory T and B cells. Our study also revealed novel subtypes in NK cells, T and B lymphocytes based on the t-SNE analysis of our chosen surface markers. These novel immunological T and B cell subtypes also showed varying degree of physical interaction with the platelets. These study open new possibilities of development of disease marker and therapeutic approaches as evidenced by previously ongoing clinical trials exploiting platelets and monocyte interaction in diseases such as HIV and CVID.

P.E3E4.01.21

MAE versus IP: a comparison of two methods of HLA class I peptide isolation

R. Farriol Duran[1,2], *G. Janssen*[2], *A. de Ru*[2], *Y. Arribas*[1], *L. Labeur*[1], *D. Jaraquemada*[1], *P. Van Veelen*[2];
[1]*Institute of Biomedicine and Biotechnology, Autonomous University of Barcelona, Cerdanyola del Vallès, Spain,* [2]*Center for Proteomics and Metabolomics, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands., Leiden, Netherlands.*

The most commonly applied method for HLA Class I peptide isolation is immunoprecipitation (IP). However, the method has a limited yield, which affects the HLA-peptidome coverage, requiring relatively large amounts of starting material (cells or tissues) to obtain a deep HLA-peptidome. Alternatively, mild acid elution (MAE) is the other established technique that can be applied. MAE is based on a citrate shock that disrupts HLA Class I complexes liberating peptides to the supernatant. The simplicity and time-efficiency of MAE comes at the expense of the (co)isolation of non-HLA-binding peptides. Our current experiments showed a relatively high fraction of HLA-binding peptides (77%), higher than generally reported, but not as good as IP (90%). Parallel elutions (with IP and MAE) of the EBV-transformed B cell line JY showed a similar HLA-binding affinity distribution for MAE and IP-derived peptides, which seems to imply that not many weak binders are lost during IP.

P.E3E4.01.22

TotalSeq™ , Standardized oligonucleotide barcode antibody conjugates for multiplex immunophenotyping by single cell sequencing

M. Li*, B. Yeung, K. Nazor, X. Zhao, X. Yang;*
BioLegend, San Diego, United States.

The combination of nucleic acid sequencing and protein expression profiling is a recent advance in single cell sequencing (scSeq) technologies. For instance, the CITE-Seq or REAP-Seq combined measurements of cellular proteins and transcriptomes. This advance will potentially transform how complex cell populations are studied. Published data, as well as those from our own studies, indicated that scSeq analysis on cell surface marker expression are comparable to multi-color flow cytometry, but provides superior capacity in multiplexing to the latter. To support application of the new technologies and enable reliable comparison of data across longitudinal and multi-site studies, we provide standardized oligonucleotide barcode-labeled antibodies. After assigning a unique oligo barcode to each of our monoclonal antibodies, we prepared the conjugated reagents; we name it TotalSeq™ product line. In addition, we attempted to assign/conjugate a series of oligonucleotide barcodes to select clones of antibodies specific to "universally" expressed cell surface molecules. These products , the Hashtags, can be used to label cells from different sources, or experiments, etc, in multiplex analysis. We will show data from our studies validating the application of these products.

P.E4.01 Cell communication and signaling in the immune system

P.E4.01.01

The dopaminergic agonist Pramipexol enhances regulatory T cell response after one-year treatment in Parkinson disease patients

L. Adalid-Peralta[1], *D. D. Álvarez Luquín*[1], *A. Arce Sillas*[1], *J. Leyva Hernández*[1], *E. Montes Moratilla*[1], *V. Vivas Almazán*[1], *C. Pérez Correa*[1], *U. Rodríguez Ortiz*[1], *M. C. Boll*[2], *G. Fragoso*[3], *E. Sciutto*[3];
[1]*Instituto Nacional de Neurología y Neurocirugía, Mexico DF, Mexico,* [2]*Instituto Nacional de Neurología y Neurocirugía, Mexico City, Mexico,* [3]*Instituto de Investigaciones Biomédicas, UNAM, Mexico City, Mexico.*

Introduction: Parkinson disease (PD) is the second most frequent neurodegenerative disease, only after Alzheimer. PD is characterized by the loss of dopaminergic neurons in the substantia nigra and by the presence of α-synuclein aggregates. PD-associated neuroinflammation contributes to neurodegeneration. The dopaminergic agonist Pramipexol has shown a neuroprotective effect by mechanisms yet unknown. Meanwhile, both inflammatory and anti-inflammatory immune cells could be stimulated by dopamine and dopaminergic agonists. Regulatory T cells (Tregs), critical in restraining inflammation, express dopamine receptors. This work is aimed to determine the effect of Pramipexol on Treg-mediated immunoregulatory responses.
Materials and Methods: 30 untreated PD patients (scoring 1-2 in the H&Y scale) and 22 healthy controls were included. Peripheral blood samples were taken at inclusion and one year after treatment with pramipexol, either alone or combined with levodopa. The levels of classical CD4+CD25+FOXP3+CD127low/-, resting CD4+CD45RO-FOXP3low, non-Treg CD4+CD45RO+FOXP3med, activated CD4+CD45RO+FOXP3hi, Tr1 CD4+CD25hiIL-10+, and Th3 CD4+CD25hiTGF-beta+ Treg cells were determined. Patient status was assessed by the UPDRS and H&Y scales.

Results: At inclusion, PD patients showed significantly lower levels of classical, activated, and Tr1 Tregs than controls. No post-treatment differences were found in these populations between patients and controls. The levels of classical, activated, and Tr1 cells showed a significant increase in patients after treatment. A decrease in UPDRS and H&Y scores was also observed.
Conclusions: The increase in Treg levels promoted by treatment suggests a recovery in the regulatory immune response that could contribute to suppress neurodegeneration-related inflammation. Treg recovery coincided with decreased UPDRS and H&Y scores.

P.E4.01.02
Binding between recombinant Phospholipases D from the venom of Loxosceles laeta and "Lipid Rafts" from the membrane of human monocyte THP-1 activates the PI3k /Akt pathway.

***T. A. Arán-Sekul**, J. M. Rojas, J. E. Araya, A. Catalán;*
Universidad de Antofagasta, Antofagasta, Chile.

Introduction: The Lipid Rafts are dynamic complexes, located on the cell surface, mainly composed of cholesterol, sphingolipids and proteins. It's believed that its most important role is to participate in the transduction of signals into the cell. Studies in mouse T cells have shown an association between the Lipid Rafts and the PI3k /Akt pathway, facilitating membrane recruitment, and activation of Akt. **Aim**: Demonstrate that the association between recombinant Phospholipases D of the venom *Loxosceles laeta* and Lipid Rafts of the monocyte membrane is able to activate the PI3k/Akt pathway. **Methodology**: Using recombinant isoforms of the *Loxosceles laeta* venom, the interaction between our proteins with Lipid Rafts of the monocyte THP-1 membrane was evaluated by immunofluorescence. Also, activation of the PI3k/Akt pathway was evaluated by Western Blot of proteins from THP-1 cells incubated with our recombinant proteins. **Results**: From the incubation with antibodies specific for Lipids Rafts, recombinant Phospholipases, marked with fluorophores, it was possible to demonstrate co-localization between our recombinant proteins and the Lipid rafts from THP-1 cells. . Also, this cell line, when incubated with recombinant Phospholipases, exhibits activation of PI3k/Akt pathway evaluated by Western Blot.**Conclusion**: No previous report has addressed the possible association of Lipid Rafts to the signals induced by Phospholipases D of *Loxosceles* spiders. In addition, its role in the activation of the PI3k/Akt signaling pathway related to the production of cytokines and chemokines associated with the inflammatory response to *Loxosceles* venom its unknown. So, according to the results, our work contributes to the understanding of this problem.

P.E4.01.03
Polymorphism analysis of TLR7 (rs179008) and TLR9 (rs352140) genes in systemic lupus erythematosus patients

***M. A. Bashir**, N. Afzal, R. Tahir, F. Shahzad, K. Javaid, M. Kashif, S. Jahan;*
University of Health Sciences, Lahore, Pakistan.

Background: Systemic Lupus Erythematosus (SLE) is an inflammatory autoimmune disease characterized by production of autoantibodies and subsequent damage to multiple organs. In SLE, various antibodies are formed and mostly anti-dsDNA levels are raised in serum of SLE patients. Various genome wide studies have shown association of TLR7 and TLR9 genes with SLE. Therefore, this study was designed to determine and compare single nucleotide polymorphism (SNP) at restriction sites of TLR7 gene (rs179008) and TLR9 gene (rs352140) between local population of SLE patients (Group-1) and healthy controls (Group-2). **Method:** It was a case control study. Eighty samples were recruited for each of the two study groups. Three ml of EDTA blood from patients and control was collected and processed for the analysis of gene polymorphism of TLR7 (rs179008) and TLR9 (rs352140) by PCR-RFLP after DNA extraction. Chi-square test was used to analyze polymorphism analysis and allele frequencies between two groups. Associations of TLR 7 and TLR 9 gene polymorphism with SLE and with its clinical parameter were analyzed. **Results:** In TLR7 genotype AT and TT are not significantly associated with SLE while TLR9 CT genotype and TT genotype and especially T allele are significantly associated with SLE showing significant interdependence of TLR9 gene polymorphism with SLE patients. **Conclusion:** Genetic variation in TLR9 may be a key part in pathogenesis of SLE; therefore and TT genotype and T allele is associated with SLE so further studies are needed to establish this genetic factor as biomarker for local population.

P.E4.01.04
N-3PUFAs reduce CD4+ T-cell distribution to adipose tissue via both cellular & systemic effects

***D. Cucchi**[1], M. D. Camacho-Munoz[2], J. Smith[3], A. Nicolaou[2], C. Mauro[1,4];*
[1]William Harvey Research Institute, London, United Kingdom, [2]Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, United Kingdom, [3]The Institute of Cancer Research, London, United Kingdom, [4]Institute of Inflammation and Ageing, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom.

The lipid imbalance observed in cardiovascular metabolic disorders (CVMD) alters T-cell membrane lipid composition, mediator production and related signalling cascades, leading to unwanted inflammatory responses. We postulated that these alterations may be corrected by omega-3-polyunsaturated fatty acids (n-3PUFAs). Indeed we show that exposure of activated T-cells to bioactive lipids can modify migratory patterns in vitro and in vivo and we investigate their mechanisms of actions. In mice fed n-3PUFA-enriched diet for 3 weeks, we found a significant reduction in the numbers of pro-inflammatory CD4+ T-cells in adipose tissue, as well as significant changes in the lipids profile in plasma and adipose tissues, consistent with an anti-inflammatory signature. To better understand the effect of n-3PUFA on T-cell motility, we assessed CD4+ T-cell migratory capabilities in vitro upon treatment with EPA and DHA in a trans-endothelial migration assay, observing a significant reduction of migration. Furthermore, when T-cells were pre-treated with EPA and DHA in vitro and then implanted in mice, they migrated to the inflamed peritoneum at much lower numbers. In line with this, we found that EPA and DHA treatments are able to reduce the number of polarised T-cells in vitro, alter membrane microdomains and decrease the activity of small Rho GTPases, such as Rhoα and Rac1, whose role in cytoskeletal dynamics is crucial. These findings show that the two principle n-3PUFA, EPA and DHA are active in reducing the motility of CD4+ T cells and their ability to reach target tissues through changes in the cytoskeleton.

P.E4.01.05
Exploring miR-34c-5p modulation of effector CD4 T cell differentiation through logical modelling

***N. Domingues**[1], F. C. Pinto[1], A. E. Sousa[2], M. Gama-Carvalho[1];*
[1]BioISI - Biosystems and integrative sciences institute, Faculty of Sciences, University of Lisbon, Lisbon, Portugal, [2]Molecular Medicine Institute, Faculty of Medicine, University of Lisbon, Lisbon, Portugal.

miRNAs are essential for proper immune cell development and function. miR-34c-5p is upregulated in naïve CD4 T cells 72 hours after in vitro stimulation, but its role in activated T cells remains unknown. The already complex signalling aspects of the immune system are exacerbated by miRNA regulation. Modelling approaches can be extremely useful for immunologists to predict system behaviours.
To address the functions of miR-34c-5p in CD4 T cell activation and differentiation, available logical models were expanded to include miRNA regulation. Validated and predicted targets for miR-34c-5p and miR-155-5p were taken from databases and acquired through prediction algorithms. Transcription factor regulation for these miRNAs was retrieved, including TSSs and TFBSs for promoter regions. Based on the obtained data, a new model was constructed to simulate the potential effects of miR-34c-5p in anti-CD3 and anti-CD28 stimulated naive CD4 T cells in the presence of IL2, using an asynchronous updating scheme. Using a specific set of logical rules, we identified GATA3, FOS and MYC, in addition to miR-34c described TFs TP53 and FOXO3, as the most likely modulators of miR-34c transcription given their ability to reproduce the experimentally observed miRNA expression patterns. Our results further suggest that miR-34c is expressed during effector Th2 differentiation.
Our in silico modelling approach provides new insights into the potential functions of miR-34c-5p in TCR-stimulated T cells, suggesting a role in the promotion of anergy or subtype plasticity, which we are currently validating experimentally.

P.E4.01.06
The role of integrins in serine protease activated protein C signalling in T-cell

***D. GUPTA**[1], S. Ranjan[1], S. Kohli[1], R. Rana[1], A. Müller[2], B. Schraven[2], B. Isermann[1];*
[1]Department of Clinical Chemistry and Pathobiochemistry, Magdeburg, Germany, [2]Institute for Molecular and Clinical Immunology, Magdeburg, Germany.

Introduction: The serine protease activated protein C (aPC) is an anticoagulant, which conveys intracellular signals *via* protease activated receptors (PARs). The function of aPC in innate immunity through both its anticoagulant and signalling properties is established. Using a mice model of GvHD, we have recently established a function of aPC and one of its receptors (PAR3) in adaptive immunity. Integrin activation and signaling has been shown to regulate T-cell activation and adhesion. Here we test the hypothesis if aPC regulates T-cell activation and adhesion via integrins on T-cells.
Material and Methods: GvHD was induced in APC^{high}, RGE-APC^{high} and C57BL/6 (WT) mice by transplanting $2x10^6$ T-cells with $5x10^6$ bone marrow cells from BALB/c mice. Survival, clinical scores and apoptosis in gut were used as parameters to study disease severity. T-cell activation,adhesion and trans-migration were studied *in vitro*. The effect of aPC on integrin signaling was studied using immunoblotting.
Results: *In-vivo* we observed that mice with endogenous high levels of aPC (APC^{high}) were protective against GvHD compared to WT mice. However, mice expressing a mutant aPC lacking the integrin binding site (RGE-APC^{high}) failed to be protective indicating that interaction of aPC with integrin is required for its immunomodulatory function in GvHD. *In-vitro,* aPC preincubation reduced T-cell activation, adhesion to ICAM1 and fibronectin, trans-migration in the presence of SDF-1 and phosphorylation of FAK and Ezrin.
Conclusion: These data demonstrate that aPC modulates T-cell activation and adhesion. *In vivo* results suggest that aPC interacts with integrins to mediate its protective effect in GvHD.

P.E4.01.08

The role of ROS hyperproduction in murine model of autoinflammatory osteomyelitis

J. Kralova[1], *A. Drobek*[1], *J. Prochazka*[2], *F. Spoutil*[2], *D. Glatzova*[1,3], *S. Borna*[1], *P. Angelisova*[1], *T. Skopcova*[1], *J. Pokorna*[1], *R. Sedlacek*[1,2], *T. Brdicka*[1];
[1]*Institute of Molecular Genetics of the ASCR, Prague, Czech Republic, Prague 4, Czech Republic,* [2]*Czech Centre for Phenogenomics, hosted by the Institute of Molecular Genetics ASCR, Vestec, Czech Republic,* [3]*J. Heyrovsky Institute of Physical Chemistry ASCR, Prague, Czech Republic.*

PSTPIP2 is an adaptor protein expressed in the myeloid cells and its deficiency leads to the development of auto-inflammatory disease in mice. This disease has been designated chronic multifocal osteomyelitis (CMO) as the main manifestation is sterile inflammation of the bones accompanied by inflammation of the soft tissues in the external parts of the body. Enhanced production of pro-inflammatory cytokine IL-1β by murine neutrophils is the main factor driving CMO development and progression. In addition, hyper-activation of various signaling pathways in CMO neutrophils has been observed. The negative regulatory effect of PSTPIP2 protein on signaling pathways and IL-1β production is likely mediated by its interacting partners. These include inhibitory molecules, such as CSK, SHIP1 and protein tyrosine phosphatases from the proline-, glutamic acid-, serine- and threonine-rich (PEST) family. However, the exact mechanism of how PSTPIP2 inhibits signaling is not known. Here we describe deregulated ROS production in CMO bone marrow cells and purified neutrophils. To investigate the role of ROS in CMO disease development and progression, CMO mice with non-functional phagocytic NADPH oxidase (Nox-2$^{-/-}$) were generated. Detailed analysis of this mouse strain including disease free curves, CT scans and IL-1β production was performed and data will be presented showing partial alleviation of the disease symptoms.
Acknowledgements: This study was supported by GACR (project number 17-07155S)

P.E4.01.09

RNAseq for novel sialic acid inhibitory signalling via Siglecs in moDC

J. Lübbers[1], *E. R. Li*[1], *A. Gallagher*[1], *S. C. Bruijns*[1], *D. Molenaar*[2], *S. van Vliet*[1], *Y. van Kooyk*[1];
[1]*VU University Medical Center, Amsterdam, Netherlands,* [2]*VU, Amsterdam, Netherlands.*

Cancer cells and pathogens, use sialic acids to actively inhibit the immune system by binding Sialic-acid binding immunoglobulin type lectins (Siglecs) with an ITIM motif. This motif becomes phosphorylated; SHP binds and phosphorylates downstream targets, triggering inhibitory processes. In mice bone marrow-derived DC, we have shown that sialic acid modified antigens are internalized and impose a regulatory program on DCs, inducing Tregs and inhibit generation of Teffector cells. To study whether similar inhibitory processes are initiated by sialic acids on human monocyte-derived DCs (moDCs), displaying Siglec 1, 7 and 9, we generated α2,3 and α2,6 sialic acids on dendrimers (sia-dendrimers). MoDC binding of these sia-dendrimers enhanced IL-10 and reduced IL-12p70 production, pointing towards a more tolerogenic phenotype. To explore the pathways induced by these sialic acids, we performed an unbiased screen of RNAseq data from moDCs treated with α2,3 and α2,6 sia-dendrimers.
α2,3 sia-dendrimer binding to moDCs uncovered 867 unique differentially expressed genes (DEGs) and α2,6 sia-dendrimer revealed 47 DEGs. Gene Set Enrichment Analysis (GSEA) of α2,3 sia-dendrimer revealed enrichment in many pathways including the high-level pathway immune system. The sub-pathways cytokine signalling and antigen processing and proteasome degradation were enriched, especially the ubiquitination sub-pathway were most pathway hubs were changed. In the α2,6 sia-dendrimer only half of the hubs in this sub-pathway were changed. This indicates that sialylated antigens are processed differently and thereby influence antigen processing and presentation leading to changes in T cell polarisation.

P.E4.01.10

Granzyme B in the cell line NK-92: novel approaches and old routines

A. V. Korenevsky, Y. P. Milyutina, A. A. Zhdanova, A. D. Shcherbitskaya, V. A. Semyonov, ***V. A. Mikhailova****, D. I. Sokolov, S. A. Selkov;*
FSBSI "The Research Institute of Obstetrics, Gynecology and Reproductology named after D.O.Ott", Saint-Petersburg, Russian Federation.

Introduction: At present, a variety of routine methods is used for the proteome evaluation. In this study, we have focused on the search for granzyme B in the cell line NK-92 using combination of novel approaches. Material and Methods: The cell line NK-92; 2D-electrophoresis (Protean i12 IEF Cell, PowerPac HC; BioRad, USA), isoelectric focusing (3100 OFFGEL fractionator; Agilent Technologies, USA), on-chip electrophoresis (bioanalyzer 2100; Agilent Technologies, USA), Western blotting. Results: The routine 2D-electrophoresis followed by MS/MS identification was accompanied by a long search for granzyme B and was no success, as staining with Coomassie had not visualized the relevant spot. Therefore, the proteins were subjected to 1D-electrophoresis followed by Western blotting to discover the target protein. The novel approach was that the cell lysate was divided into 24 soluble fractions depending on isoelectrical point, which allowed for 1D-electrophoresis followed by Western blotting and subsequent identification of the fraction containing the highest amount of granzyme B. Additionally, the protein profiles of the obtained fractions were then compared using on-chip electrophoresis. The advantage of this approach is significant timesaving, when compared to the Laemmli method. Besides, the remaining fractions allows performing straight mass-spectrometry or separating proteins using micropreparative HPLC with subsequent MS/MS identification. Conclusions: The combination of novel approaches using routine methods allow for more valid granzyme B identification in the cell line NK-92. Supported by RFBR grant #17-04-00679 and President's grant NSh-2873.2018.7. The study was performed using equipment of the SPbSU Science Park resource center "Development of molecular and cellular technologies."

P.E4.01.11

Mesenchymal stem cells from healthy human gingiva produce lower levels of IL-6 compared to their counterpart from chronic periodontitis

M. Milinković[1], *M. Markovic*[2], *I. Majstorovic*[2], *M. Milanovic*[2], *S. Zečević*[1], *S. Todorovic*[1], *M. Colic*[3];
[1]*University of East Sarajevo, Medical Faculty Foca, R.Srpska, BiH, Foca, Bosnia and Herzegovina,* [2]*University od Defence in Belgrade, Medical Faculty of the Military Medical Academy, Belgrade, Serbia, Belgrade, Serbia,* [3]*University of East Sarajevo, Medical Faculty Foca, R.Srpska, BiH. University od Defence in Belgrade, Medical Faculty of the Military Medical Academy, Belgrade, Serbia, University of Belgrade. Institute for Application of Nuclear Energy, Belgrade, Serbi, Belgrade, Serbia.*

Mesenchymal stem cells (MSCs) have been isolated and characterized from different dental tissues, including gingiva. However, litle is know whether and how chronic inflammation changes their functions. The aim of this study was to compare phenotypic profile, differentiation potential and cytokine production between MSCs isolated from human healthy gingiva and gingiva from chronic periodontitis patients. We showed that 90-98% of both types of MSCs expressed typical markers such as CD90, CD73 and CD105 and were able to differentiate into osteoblasts, chondroblasts and adipocytes under specific cell culture conditions. The expression of other markers, including CD146, CD56, STRO-1 and PDGF-R was lower on MSCs from healthy gingiva, but their proportion in both groups was variable, depending on the donor and number of culture passages. By using fluorescent and confocal microscopy, we demonstrated that pericytes, supposed to be the dominant source of these MSCs *in vivo*, expressed strongly NG2, PDGFR, CD146, CD105 and CD166, but not CD34 and CD31, the markers of endothelial cells. The relative number of pericytes was higher in disseased gingiva. Both types of MSCs produced IL-6, but its level was significantly lower in cultures of MSCs from healthy gingiva. The addition of conditioned medium from the tissue explant of gingival biopsies from chronic periodontitis augmented the production of IL-6 by MSCs from healthy gingiva, suggesting that gingival MSCs during periodontitis , through enhanced production of IL-6, could have a proinflammatory role.

P.E4.01.12

Immunophenotyping extracellular vesicles using Amnis imaging technology

H. R. Pugsley*, B. R. Davidson, P. Morrissey;*
Merck, Seattle, United States.

Only recently has the importance of extracellular vesicles (EVs) as key mediators of intercellular communication been appreciated. EVs are membrane derived structures that include exosomes, microvesicles and apoptotic bodies. Exosomes have been shown to transfer molecules between cells and have the potential to transfer signals between cells. Exosomes are released under normal physiological conditions; however, they are also believed to serve as mediators in the pathogenesis of neurological, vascular, hematological and autoimmune diseases as well as cancer. Quantifying and characterizing EVs in a reproducible and reliable manner has been difficult due to their small size (exosomes range from 30 - 100 nm in diameter). EV analysis can be performed using high-magnification microscopy however this technique has a very low throughput. Attempts to analyze EVs using traditional PMT based flow cytometers has been hampered by the limit of detection of such small particles and low refractive index. To overcome these limitations we have employed the recently developed CellStream flow cytometer. The CellStream utilizes the Amnis imaging technology, having the advantage of high throughput flow cytometry with higher sensitivity to small particles due to the time-delay-integration image capturing system. In this study, the CellStream was used to immunophenotype EVs derived from red blood cells and platelets.

P.E4.01.13

Identifying exosome binding and internalization in blood cell subsets by multispectral imaging flow cytometry

H. R. Pugsley*, S. L. Friend, B. E. Hall, P. Morrissey;*
Merck, Seattle, United States.

Exosomes have been shown to transfer molecules between cells and have the potential to transfer signals between cells. To study how exosomes are interacting with white blood cells high-magnification microscopy can be used; however, this technique has very low throughput. In addition, these events are rare and therefore difficult to analyze objectively and statistically by traditional microscopy methods. To overcome these limitations we have employed multispectral imaging flow cytometry that has the advantage of combining high throughput flow cytometry with high sensitivity fluorescence microscopy. In this study we use multispectral imaging flow cytometry to investigate the interaction of exosomes with white blood cells. Exosomes derived from Jurkat cells were labeled with anti-human CD63-AF647 and added to human white blood cells.

The cells were labeled for immunophenotyping, fixed, and then labeled with anti-human CD63-PE to identify external exosomes.
Plotting Internalization vs Bright Detail Similarity facilitated the identity 3 populations: Internal Exosomes, External/Internal Exosomes, and External Exosomes. Neutrophils, monocytes, and lymphocytes were identified by immunophenotyping; the % of each blood cell subset associated with the CD63-AF647 labeled exosomes and whether the exosomes were internalized or external was investigated. The monocytes had the highest % of cell associated with CD63-AF647 labeled exosomes. And in all of the cell types the majority of the cells associated with CD63-AF647 labeled exosomes were either internalized or partially internalized.

P.E4.01.14

Establishment of an engineered antigen presenting cell platform to study antigen specific human CD8 T cell responses *in vitro*

M. Reithofer[1]*, S. Rosskopf*[2]*, C. Battin*[3]*, J. Leitner*[3]*, B. Bohle*[4]*, B. Jahn-Schmid*[4]*, P. Steinberger*[3]*;*
[1]*Institute for Pathophysiology and Allergyresearch, MCCA PhD Programme, Medical University, Vienna, Austria,* [2]*Institute of Immunology, MCCA PhD Programme, Medical University, Vienna, Austria,* [3]*Institute of Immunology, Medical University, Vienna, Austria,* [4]*Institute for Pathophysiology and Allergyresearch, Medical University, Vienna, Austria.*

Studies on antigen-specific human CD8 T cells are hampered by their low frequency in the peripheral blood. Expansion of these cells in vitro by antigenic peptide is inefficient and often precludes a thorough characterization. Here we describe a cellular platform which allows to efficiently stimulate and expand antigen-specific human CD8 T cells. HLA-A201, the most common MHC class I molecule in the Western population was expressed on human K562 cells. In addition, we introduced an artificial gene encoding five major HLA-A2 restricted viral peptides targeted into the proteasomal degradation and antigen processing pathway. Upon coculture with PBMCs of HLA-A2 positive human donors, these eAPC efficiently expanded virus-specific T cells. Additionally generated eAPC expressing costimulatory ligands like CD80, CD86 or 4-1BBL had augmented capacity to stimulate antigen-specific T cells. Cytotoxicity assays confirmed that stimulation with eAPC yielded potent CD8 effector T cells. MHC class I tetramer staining experiments were performed to identify stimulation conditions that promoted the expansion of antigen-specific T cells and prevented bystander T cell activation. Taken together, our results indicate that we successfully established an eAPC platform that allows to evaluate the function of accessory molecules in the expansion of antigen-specific human $CD8^+$T cells. This system may be applied for the development of improved protocols for *in vitro* generation of virus and tumour-specific T cells for adoptive therapy.

P.E4.01.15

Analysis of miRNA involvement in CD4+T cell differentiation

G. A. Rockinger[1]*, P. Roelli*[2]*, D. Zehn*[2]*, G. Scholz*[3]*, P. Romero*[1]*, C. Jandus*[1]*;*
[1]*University of Lausanne, Epalinges, Switzerland,* [2]*Technical University of Munich, Munich, Germany,* [3]*University Hospital of Bern, Bern, Germany.*

Introduction: in contrast to CD8+T cell, CD4+ T have only recently gained increasing importance in tumor immunity as studies showed their therapeutic relevance, including the recognition of neo-antigens. A key to CD4+ T cell usage in immunotherapy will depend on a better understanding of the regulation of CD4+ T cell differentiation, to promote stem cell memory (SCM) and central memory (CM) phenotypes.
Material and methods: we performed a mRNA sequencing and a microRNA (miR) array of highly-pure sorted naïve (N), SCM, CM and effector memory (EM) CD4+ T cell subsets from peripheral blood of 4 healthy donors, followed by in depth Bioinformatic analysis and in vitro target validation.
Results: we identified differential expression between N, SCM, CM and EM cells of known miR such as miR-146a-5p and miR-155-5p and of previously undescribed miR. Further investigations in additional healthy donors' samples confirmed by qPCR the differential expression of these miR. Further, we were able to correlate the expression of candidate miRs with up or downregulation of target genes within the CD4+ T cell subset of interest.
Conclusion: we are presently investigating miR and target mRNA expression in vitro and in vivo using TCR transgenic mouse models. We aim at identifying optimal miR candidates that could be therapeutically targeted to influence the differentiation of a Naïve CD4+T cells into SCM or CM CD4+ T cells capable of targeting tumor cells.

P.E4.01.16

T cell cooperativity shapes antigen-specific immune responses

S. Zenke[1]*, J. Braun*[1]*, J. Beltman*[2]*, A. Gavrilov*[3]*, N. Beyersdorf*[4]*, P. Aichele*[5]*, T. Lämmermann*[3]*, T. Schumacher*[6]*,* ***J. Rohr***[1]*;*
[1]*Center for Chronic Immunodeficiency, Freiburg, Germany,* [2]*Leiden Academic Centre for Drug Research, Leiden, Netherlands,* [3]*Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany,* [4]*Institute for Virology and Immunobiology, Würzburg, Germany,* [5]*University Medical Center Freiburg, Institute of Immunology, Freiburg, Germany,* [6]*The Netherlands Cancer Institute, Amsterdam, Netherlands.*

Upon activation T lymphocytes rapidly aggregate and form cell clusters both *in vitro* and *in vivo* in an LFA1:ICAM1 dependent manner. The close proximity of cells within clusters provides ample opportunity for T cells to communicate their activation state to each other – via paracrine messengers and/or direct cell interaction. We hypothesized that within clusters activated T cells mutually regulate their behaviors and subsequent differentiation akin to quorum-regulation of bacteria. In order to identify molecules involved in such crosstalk, we have developed a bioinformatics-approach allowing us to identify receptor-ligand pairs co-expressed on activated T cells. We then characterized selected interactions and revealed that within clusters cellular crosstalk sustains T cell expansion by limiting activation-induced cell death. Furthermore, we found evidence for an additional related counter-regulatory signaling circuit which limits further T cell expansion. Competition between these two signaling circuits shapes the outcome of T cell activation. In summary, our results indicate that T cell clusters serve as hubs for mutual regulation of activated T cells. Such behavioral coordination within a defined spatiotemporal framework enables the generation of robust yet flexible immune responses at the population level.
Funded by DFG-SFB1160

P.E4.01.17

***Loxosceles' spider* phospholipases D activates the PI3K/Akt signaling pathway, and join to lipid raft present in human skin fibroblasts.**

J. M. Rojas Morales*, T. Arán Sekul, J. E. Araya, A. Catalán;*
Universidad de Antofagasta, Antofagasta, Chile.

Introduction: Phospholipase D (PLD) family enzymes from *Loxoceles* spider venom, are responsible of dermonecrosis during human spider envenomation. On human skin fibroblasts, different recombinant isoforms of PLDs from *L. laeta*, induced IL-6, IL-8, CXCL1 and CCL2/MCP-1 production. However, the signaling pathway implicated in this process is unknown. **Aim:** Evaluate the signaling pathway involved in chemokines production mediated by *Loxosceles* PLDs on skin fibroblast. **Methodology:** Human skin fibroblasts HHF-1 cultures were incubated with recombinant PLDs from *L. laeta* (rLlPLD1 and rLlPLD2), and the mutant isoforms of rLlPLD1 (rLlPLD1-D259G and rLlPLD1-W256S), and the Akt activation were evaluated by western blot, using an anti-phospho-Akt1/PKBα (Ser473) monoclonal antibody. Additionally, PI3K participation was determined by Western Blot of phosphorylated Akt in presence or absence of PLDs plus wortmannin. The PLDs-lipid raft binding was evaluated by immunofluorescence using kit Vybrant® Lipid Raft labeling kit (Molecular Probes Inc,). The phospholipase D activity of rPLDs was determinates by Amplex Red Sphingomyelinase Assay.
Results: The recombinant PLDs from *L. laeta* activate Akt between 5-15 min in fibroblast cultures. This effect was decreased in cultures treated with rLlPLDs and wortmannin. Also, the substrate deficient rLlPLD1 mutants, showed a lower activation of PI3K/Akt pathway compared to native protein. Also, PLD binding to lipid rafts from fibroblasts plasmatic membrane seem to be involved in PI3K/Akt pathway activation. **Conclusion:** PI3K/Akt signaling pathway is activated by rLlPLDs, involved binding to lipid rafts present in the plasma membrane of skin fibroblasts, suggesting a role of the latters in the chemokines expression during *Loxosceles* envenomation.

P.E4.01.18

Lymphocyte-specific tyrosine-protein kinase Lck homo-dimers in the complex control of T-cell receptor signaling

P. Schatzlmaier[1]*, F. Baumgart*[2]*, P. Eckerstorfer*[1]*, S. Kraupp*[1]*, G. Schütz*[2]*, H. Stockinger*[1]*;*
[1]*Medical University of Vienna, Vienna, Austria,* [2]*Vienna University of Technology, Vienna, Austria.*

Engagement of the T-cell antigen receptor (TCR) initiates a signaling cascade resulting in T-cell activation, proliferation and differentiation. Intracellular lymphocyte-specific kinase (Lck) plays a pivotal role in this process, transducing initial TCR/CD3 stimulation into tyrosine phosphorylation, calcium fluxing, synapse formation, and altered gene expression. Lck activity is regulated on multiple intercalated levels, including its subcellular localization (by transporters), 2D nano-domain distribution within the plasma membrane, and its phosphorylation status that is directly linked to its enzymatic activity. A potential mechanism of Lck regulation not investigated so far is its homo-dimerization. Noteworthy, ligand-induced homo-association followed by trans-activatory auto-phosphorylation is an established principle for transmembrane receptor tyrosine kinases.
Employing a super-resolution imaging technique - Thinning Out Clusters while Conserving the Stoichiometry of Labeling - we identified a significant amount of Lck dimers in living T-cells. Furthermore, homo-association of membrane-anchored Lck was confirmed by co-immunoprecipitation. To investigate the role of Lck homo-dimers in T-cell signaling, we established an inducible Lck-dimerization system in human Jurkat T-cells after CRISPR/Cas9 knock-out of endogenous Lck. Controlled and specific dimerization of Lck by a membrane-permeable X-linking agent significantly altered its phospho-status and enzymatic activity in a titratable fashion, modulating early as well as late TCR signaling events. In conclusion, homo-dimerization of Lck represents a novel regulatory mechanism controlling Lck kinase activity and thus stimulatory thresholds for T-cell activation.

P.E4.01.20

HyperIgM syndrome caused by defect in CD40

N. kechout[1], *S. Khalissa*[1], *L. Smati*[2], *R. Yahi*[1], *S. Otsmane*[1], *F. Benhassine*[2], *N. Attal*[1];
[1]*Institut Pasteur d'Algérie, Algiers, Algeria,* [2]*Department of Pediatrics, EPH Bologhine, Algiers, Algeria.*

Introduction: Hyper IgM Syndrome (HIGM) is a rare primary immunodeficiency with defects in immunoglobulin (Ig) class switch recombination . So, the affected patients have low levels of IgG and IgA and normal or elevated IgM, resulting in high susceptibility to infections. This defect can be caused by alteration in T-B (CD40-CD40L) interaction or by intrinsic B cell abnormalities. Germinal center can not be formed. The most common form is X-linked inherited and is due to mutations in CD40 ligand (CD40L) gene. In this study we report the first case of Algerian patient with HIGM caused by defect in CD40.
Materials/Methods: A male patient aged of 10 months referred us for exploration. Immunological techniques used are: measurement of IgG, IgA and IgM by nephelometry; T, B, NK immunophenotyping , evaluation of CD40, CD40 L expression and numeration of CD27 + memory B cells by flow cytometry.
Results: The patient had a medical history of pneumonia, recurrent otitis until 8 months. Studying his medical documents revealed an episode of Pneumocystis jiroveci pneumonia. He also suffer from persistent oral candidiasis. He has low levels of IgG, IgA, normal levels of IgM and normal expression of CD40L. Furthermore, he has decreased memory B cells and lack of CD40 expression on monocytes and B lymphocytes.
Conclusion: The clinical phenotype of our patient is that of a combined immunodeficiency similar to patients with CD40L defect. Lack of CD40 enables us to confirm the diagnosis of hyper IgM syndrome and consequently its autosomal recessive inheritance.

P.E4.01.21

Toll-like receptor activation by lipid nanoparticle delivered messenger RNA

S. Bates[1], *M. Ingelsten*[2], *M. Beano*[1], *M. Fellows*[2], *N. Gay*[3], *C. Betts*[1];
[1]*Pathology, Drug Safety and Metabolism, IMED Biotech Unit, AstraZeneca, Cambridge, United Kingdom,* [2]*New Modalities, Drug Safety and Metabolism, IMED Biotech Unit, AstraZeneca, Cambridge, United Kingdom,* [3]*Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom.*

Lipid nanoparticles (LNPs) are the most developed delivery formulations for messenger RNA (mRNA) therapy. They aid cellular delivery of mRNA and prevent extracellular degradation with proven efficacy, both *in vivo* and *in vitro*. A key safety issue is the activation of an innate immune response. Understanding the cellular mechanisms and activated inflammatory pathways are key to improving mRNA/LNP design, and hence their therapeutic potential. Here we investigate if mRNA/LNP delivery activates pattern recognition receptors such as Toll-like receptors (TLRs). Primary human dermal fibroblasts were treated with different TLR and cellular pathway inhibitors 30 mins prior to LNP treatment for 24 hours. The effect on proinflammatory cytokines and exogenous mRNA protein expression (luciferase) were measured. Of eight cytokines assessed, only G-CSF, IP-10, IL-8 and IL-6 were detectable. Whilst TLR4 inhibition did not impact cytokine release induced by this LNP, inhibition of downstream proteins such as IRAK4 and MyD88 led to significant drops in cytokine levels. Additionally, inhibition of endosomal formation by dynamin inhibition resulted in the largest drop in all detected cytokines, suggesting that endosomal uptake of LNP/mRNA is required to induce inflammation. This initial data supports a role for TLR signaling in mRNA/LNP activation of fibroblasts and work is ongoing to further define the mechanisms of activation and the specific TLR receptors involved.

P.E4.01.22

The ERBB-STAT3 Axis Drives Tasmanian Devil Facial Tumor Disease

L. Kosack[1], *A. Popa*[1], *B. Wingelhofer*[2], *A. Orlova*[2], ***B. Agerer***[1], *B. Vilagos*[1], *P. Majek*[1], *K. Parapatics*[1], *A. Lercher*[1], *A. Ringler*[1], *J. Klughammer*[1], *M. Smyth*[1], *K. Khamina*[1], *H. Baazim*[1], *D. A. Rosa*[3], *J. Park*[3], *P. T. Gunning*[3], *C. Bock*[1], *H. V. Siddle*[4], *S. Kubicek*[1], *E. P. Murchinson*[5], *K. L. Bennett*[1], *R. Moriggl*[2], *A. Bergthaler*[1];
[1]*CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria,* [2]*Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Vienna, Austria,* [3]*University of Toronto, Toronto, Canada,* [4]*Department of Biological Science, University of Southampton, Southampton, United Kingdom,* [5]*Transmissible Cancer Group, Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom.*

The marsupial Tasmanian devil (Sarcophilus harrisii) faces extinction due to transmissible devil facial tumor disease (DFTD). To unveil the molecular underpinnings of DFTD, we designed an approach that combines sensitivity to drugs with an integrated systems-biology characterization. Sensitivity to inhibitors of the ERBB family of receptor tyrosine kinases correlated with their overexpression, suggesting a causative link. Proteomic and DNA methylation analyses revealed tumor-specific signatures linked to oncogenic signaling hubs including evolutionary conserved STAT3. Indeed, ERBB inhibition blocked phosphorylation of STAT3 and arrested cancer cells. Pharmacological blockade of ERBB signaling prevented tumor growth in a xenograft model and resulted in recovery of MHC class I gene expression. This link between the hyperactive ERBB-STAT3 axis and MHC class I mediated tumor immunosurveillance provides mechanistic insights into horizontal transmissibility and led us to the proposition of a dual chemo-immunotherapeutic strategy to save Tasmanian devils from DFTD.

P.E4.01.23

The Differentiation of Human Amniotic Fluid Mesenchymal Stem Cells into Cardiomyocyte-Liked CellsThe Differentiation of Human Amniotic Fluid Mesenchymal Stem Cells into Cardiomyocyte-Liked Cells

S. Aungsuchawan[1], *R. Markmee*[1], *P. Pothacharoen*[2], *W. Tancharoen*[1], *S. Narakornsak*[1], *T. Laowanitwattana*[1];
[1]*Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand,* [2]*Thailand Excellence Center for Tissue Engineering and Stem Cells, Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.*

The purpose of this study was attempted to evaluate the efficiency of ascorbic acid (AA) on the effects on cardiomyogenic differentiation of human amniotic fluid mesenchymal stem cells (hAF-MSCs). The result of immunofluorescence and immunoenzymatic staining of the AA combined with 5-aza treatment group revealed the highest expression of cardiac specific proteins including GATA4, cTnT, Cx43 and Nkx2.5. It could be concluded that AA might be a cardiomyogenic inducing factor for mesenchymal stem cells and may open new insights into future biomedical applications for cardiomyogenic differentiation.

P.E4.01.24

Inhibitory of Rhinacanthus nasutus (L.) Kurz leaf extract on melanogenesis in B16F10 melanoma cells

B. Pratoomthai[1], *W. Gangnonngiw*[2,3], *J. Naowaboot*[4], *T. Songtavisin*[5];
[1]*Department of Basic Medical Science, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand,* [2]*Centex Shrimp, Faculty of Science, Mahidol University, Bangkok, Thailand,* [3]*National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency, Pathum Thani, Thailand,* [4]*Division of Pharmacology, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand,* [5]*Division of Anatomy, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand.*

Hyperpigmentation of the skin results from excessive melanin formation in melanocytes, and over production of melanin frequently leads to melanoma. The aim of this study was to investigate the potential of *Rhinacanthus nasutus* (L.) Kurz leaf water extract on inhibition of melanin formation or melanogenesis. *R. nasutus* leaf water extract was evaluated *in vitro* for its inhibitory effect on mushroom tyrosinase activity and cellular tyrosinase activity. B16F10 mouse melanoma cells were cultured with *R. nasutus* leaf extract and their tyrosinase activity and melanin content was compared with kojic acid, a known tyrosinase inhibitor. Moreover, the level of expression of melanogenesis related genes and proteins were determined by quantitative RT-PCR and enzyme-linked immunosorbent assay (ELISA), respectively. The result showed that *R. nasutus* leaf extract had no inhibitory effect on mushroom tyrosinase activity. However, *R. nasutus* leaf extract significantly suppressed cellular tyrosinase activity and melanin production in B16F10 melanoma cells without any apparent cytotoxicity. Quantitative RT-PCR and ELISA revealed that *R. nasutus* leaf extract downregulated the expression of microphthalmia-associated transcription factor (MITF) and tyrosinase mRNA and proteins. Taken together, the data suggest that *R. nasutus* leaf extract may act as an anti-melanogenic agent by inhibiting the expression of MITF and cellular tyrosinase activity. *R. nasutus* leaf extract may show potential as an ingredient in skin-whitening cosmetics or as a topical agent for the treatment of hyperpigmentation disorders.

Author Index

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

B

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

C

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

D

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

E

F

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

G

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

H

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

I

J

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

K

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

L

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

M

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

N

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

O

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

P

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

Q

R

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

S

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

T

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

U

V

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

W

Author Index

Note: Bold presentation numbers indicate that the author is the presenting author of this abstract.

X

Y

Z